University of Alberta

The formation of di- and trichloroacetic acid (TCAA and DCAA) during beverage preparation and its impact on quantitative exposure assessment for drinking water disinfection by-products

by

Justin Peter Craig Balko

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of *Master of Science*

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *The formation of di- and trichloroacetic acid (DCAA and TCAA) during beverage preparation and its impact on quantitative exposure assessment for drinking water disinfection by-products* submitted by *Justin Peter Craig Balko* in partial fulfillment of the requirements for the degree of *Master of Science* in *Medical Science – Public Health Science*.

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For My Family

Abstract

The effect of beverage preparation on trichloroacetic acid (TCAA) and dichloroacetic acid (DCAA) concentrations was assessed by analysis of water samples and samples of beverages made with the same water. Samples were analyzed by solid phase microextraction with gas chromatography using electron capture detection (SPME GC-ECD. TCAA and DCAA concentrations increased when beverages were prepared. Increasing the chlorine residual caused a corresponding increase in the concentration of TCAA and DCAA when beverages were prepared. Increase in TCAA from coffee preparation was applied to hot beverage ingestion data from a human exposure trial. Applying the elevated exposure estimates showed a considerable impact on estimated TCAA ingestion and excretion, especially for those ingesting large volumes of hot beverages. TCAA ingestion exposure estimates are affected by applying beverage data, indicating that a reliable exposure estimate should include beverage ingestion information.

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List of Abbreviations

ANOVA – Analysis of Variance

DBPs – Disinfection by-products

DCAA – Dichloroacetic acid

EPA – Environmental Protection Agency

ESI-FAIMS - MS – Electrospray Ionization, High-field asymmetric Waveform Ion Mobility Spectrometry, and Mass Spectrometry

FDA – Food and Drug Administration

GC / ECD – Gas Chromatography with Electron Capture Detection

HAA – Haloacetic Acids

HANs – Haloacetonitriles

HPLC – High Performance Liquid Chromatography

LLE – Liquid-Liquid Extraction

MDL – Method Detection Limit

MTBE – Methyl – *Tert* – Butyl Ether

NHAPS – National Human Activity Pattern Survey

NOM – Natural organic matter

RSD – Relative standard deviation
SPE – Solid phase extraction

SPME – Solid-phase microextraction

THMs – Trihalomethanes

TOC – Total organic carbon

Chapter 1: General Introduction

1.1 Scope

The emphasis of this thesis is to contribute towards a comprehensive understanding of human exposure to disinfection by-products (DBPs). The majority of DBP research has been focused on exposure from plain water obtained from public drinking water supplies, without taking into account DBP exposures from other ingestion sources or consumables prepared with tap water. With this in mind, I investigated DBP levels in *beverages* prepared with tap water (another common form of water ingestion). To date, no one has investigated the formation of DBPs (namely HAAs) in beverage preparation. Gaining this knowledge is important in order to determine if our present understanding of human exposure to DBPs is greatly affected by factoring in the DBP profile found in beverages prepared with tap water.

This thesis is broken into three major parts, with each chapter reflecting a specific objective with regard to DBPs. Chapter one provides a general overview of DBPs, and also provides further reasoning for performing research on beverages. Chapters two and three narrow the focus to HAAs (namely trichloro- and dichloroacetic acid) found in beverages. Chapter two focuses on the experimental methods implemented to determine HAA levels in water and beverages. Ultimately, this chapter provides all of the analytical data pertaining to the HAAs found in water and beverages. Chapter three focuses on the implications that the experimental data has on assessing

exposure to humans. The HAA levels observed in beverages will be applied to extensive consumption data obtained from research performed by Froese et al. (2001). Chapter three will illustrate the difference in human exposure when HAA levels are applied to ingestion data. Furthermore, a final summary chapter will serve to elucidate the implications of the discovered results and also suggest directions for further research into the subject.

1.2 Introduction

The provision of potable water requires the removal of pathogenic microorganisms and "obnoxious chemical compounds or potential toxicants" (Sarrion et al., 2000). This universally accepted concept was established in the mid-1800s by Dr. John Snow, who discovered that water supplies could effectively transmit deadly diseases such as cholera. In North America, widespread water disinfection by way of chlorination was introduced in 1908 as a means of limiting the transmission of disease in the water supply. Booker (2000) notes that the widespread water chlorination all but eliminated once common diseases such as cholera, dysentery, and typhoid fever. Karlin (1999) states that "chlorination of drinking water to prevent disease became one of the most widely practiced public health measures in the developed world and may be the most effective health measure undertaken in the twentieth century". Present knowledge suggests that this effective means of limiting disease transmission may potentially have a drawback that is manifested in the

formation of disinfection by products (DBPs). Richardson (1998) notes that "the chemical by-products produced from chlorine and other disinfectants are not completely understood, and the risk of these chemicals to human health remains a question". Disinfection by-products are the result of chlorine's potent oxidizing power causing reactions between chlorine and naturally occurring organic material in raw water (Wigle, 1998). Fawell et al. (1997) note that these by-products have been shown to produce adverse health effects in laboratory animals. With the interest of public health in mind, it is neccessary to understand the potential human health implications associated with water chlorination. While it is clear that we can not achieve a level of zero risk, increased knowledge allows us to balance the risks associated with DBPs and the risk of disease transmission through water supplies in order to minimize adverse health effects in humans.

It is clear that DBPs are present in drinking water, and the majority of epidemiological exposure assessment has focused on the ingestion of drinking water. The question that has not been addressed is whether or not the DBP concentrations are affected by the production of beverages, both hot and cold, using tap water. Tap water contains residual chlorine that is free to react with any organic compounds introduced in the making of a beverage. The focus of this work is to investigate the concentrations of trichloro- (TCAA) and dichloroacetic acid (DCAA) in tap water and in beverages made with the tap water. TCAA is currently being investigated as a potential biomarker of exposure for ingestion of DBPs. Previous studies have

estimated that approximately 35% of TCAA is lost during boiling water for three minutes for coffee or tea preparation (Kim et al., 1999). However, no studies of HAAs in the prepared beverages have been reported. Given the fact that approximately 2/3 of all water is ingested via beverages (Raymer et al., 2000), any changes to the DBP profile during beverage preparation will ultimately affect an individual's exposure to these compounds.

1.3 Disinfection By-Products

1.3.1 Overview

The reaction of chlorine with natural organic matter such as humic and fulvic acids result in the formation of a wide range of halogenated compounds (Richardson, 1998; Nieuwenhuijsen et al., 2000). These compounds have been named disinfection by-products (DBPs), and are ubiquitous in water supplies treated with chlorine. Table 1.1 provides a list of the disinfection by-products, including trihalomethanes (THMs), haloacetonitriles (HANs), haloketones (HKs), chloropicrin (CP), and haloacetic acids (HAAs) (Krasner et al., 1989). Of the DBPs that form due to chlorination, the most prevalent are the THMs and HAAs (Martinez et al., 1998; Richardson, 1998; Sarzanini et al., 1999; Weisel et al., 1999; Nieuwenhuijsen et al., 2000; Sarrion et al., 2000). To date, the majority of health research has focused on the THMs due to their relatively high quantities (Nieuwenhuijsen et al., 2000), the fact that chloroform, the major THM, was shown to be an animal carcinogen (National Cancer Institute,

1975; National Cancer Institute, 1976; Jorgenson, 1985) and the most monitoring data is available for THMs among all of the DBPs. Epidemiologic studies have shown possible associations between THM exposure and incidences of bladder cancer (King and Marrett, 1996) and rectal cancer (Hildesheim et al., 1998; Koivusalo et al., 1998). In addition, studies have shown associations of THM exposures with adverse reproductive outcomes (Kramer et al., 1992; Aschengrau et al., 1993; Bove et al., 1995; Savitz et al., 1995; Reif et al., 1996; Kanitz et al., 1996; Gallagher et al., 1998; Waller et al., 1998; Dodds et al., 1999; Klotz and Pyrch, 1999).

Increased knowledge regarding the adverse health effects linked to water disinfection has prompted research on other classes of DBPs. HAAs have also attracted regulatory attention because of their relatively high prevalence (Reif et al., 1996; Nieuwenhuijsen et al., 2000) and their potential health effects (Magnuson and Kelty, 2000; Sarrion et al., 2000). Based on a number of studies associating HAAs with adverse health effects, in 1998 the U.S. Environmental Protection Agency (USEPA) proposed 60 μg/L as a maximum contaminant level for the sum of the concentrations of five haloacetic acids (HAA5: mono-, di-, and trichloroacetic acids and mono- and dibromoacetic acids) (USEPA, 1998). Chemical structures of the nine regulated HAAs are given in Figure 1.1.

The USEPA has classified dichloroacetic acid (DCAA) as a Group B2, probable human carcinogen based on findings of liver carcinogenicity in male B6C3F1 mice

(Bull et al., 1990; DeAngelo et al., 1991) and male rats (Mather et al., 1990). In addition, trichloroacetic acid (TCAA) has been classified as a Group C, possible human carcinogen based on limited evidence linking TCAA to liver carcinogenicity in mice (Bull et al., 1990; DeAngelo et al., 1991). A number of recent epidemiologic studies have suggested a link between ingestion of chlorinated drinking water and adverse health effects, namely bladder and renal cancers (Cantor et al., 1987; Kramer et al., 1992; McGeehin et al., 1993) and reproductive and developmental effects (Bove et al., 1995; Swan and Waller, 1998; Waller et al., 1998).

With the abundance of information linking DBPs with adverse health effects, it is neccessary to obtain as much information as possible that will assist in our understanding of these chemicals and their interaction with human beings. While there are many chemical classes that fall under the designation "Disinfection By-Products" (see Richardson, 1998), the focus of this work was directed towards haloacetic acids, particularly DCAA and TCAA, in part due to concurrent research in this laboratory on the validation of urinary TCAA as a biomarker of DBP exposure.

Disinfection by-product	Highest quarterly average (μg/L)
Trihalomethanes	44
Chloroform	
Bromodichloromethane	
Chlorodibromomethane	
Bromoform	
Haloacetic acids	21
Monochloroacetic acid	
Dichloroacetic acid	
Trichloroacetic acid	
Monobromoacetic acid	
Dibromoacetic acid	
Aldehydes	6.9
Formaldehyde	
Acetaldehyde	
Haloacetonitriles	4.0
Trichloroacetonitrile	
Dichloroacetonitrile	
Bromochloroacetonitrile	
Dibromoacetonitrile	
Haloketones	1.8
1,1 – Dichloropropanone	
1,1,1 – Trichloropropanone	
Miscellaneous	
Chloropicrin	
Chloral Hydrate	
Cyanogen chloride	
2,4,6 – Trichlorophenol	

Table 1.1: Disinfection by-products and highest quarterly averages from 35 water utilities across the U.S. and California, as measured by Krasner et al. (1989). This data was taken from Nieuwenhuijsen et al. (2000).

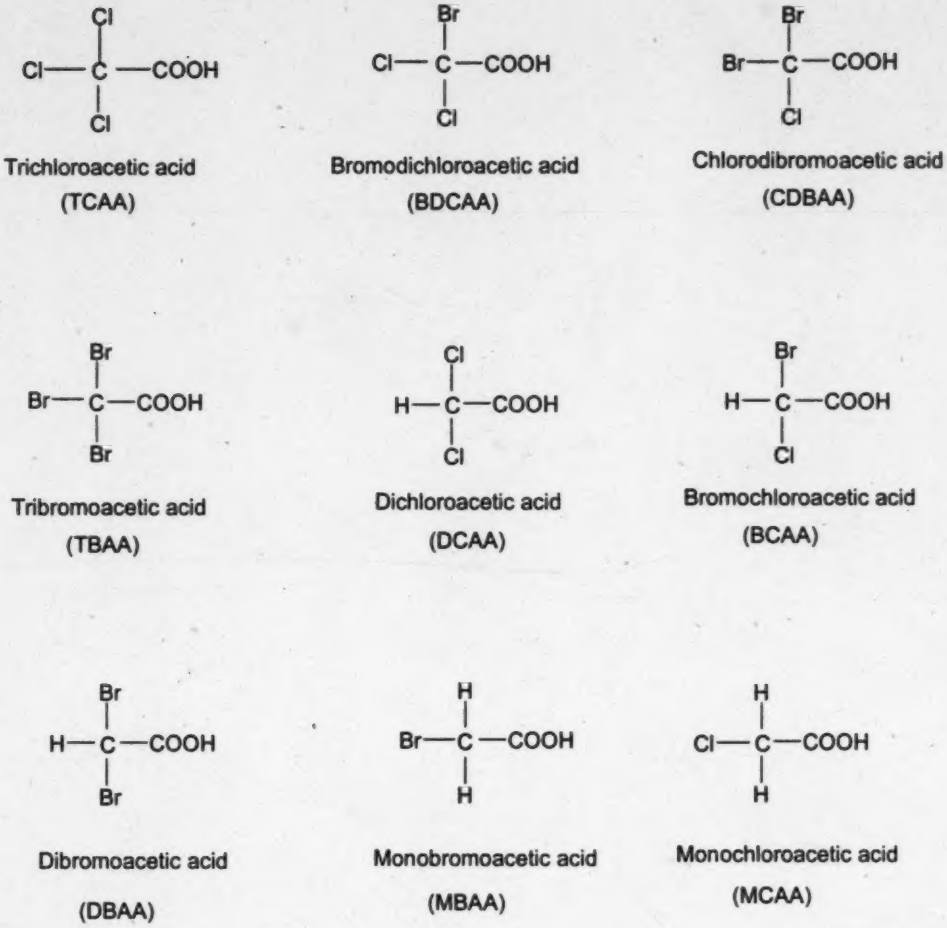


Figure 1.1: Structure of the nine haloacetic acids (HAAs) found in drinking water. The species we analyzed include trichloroacetic acid (TCAA) and dichloroacetic acid (DCAA).

1.3.2 Human Health Effects Research

1.3.2.1 Current experimental evidence

The potential adverse health effects associated with DBP exposure has prompted increasing research to better understand the human health risks associated with exposure. As stated earlier, epidemiological studies have been performed to examine the health effects produced by DBPs. While there have been a number of epidemiologic studies performed, there is still a lack of reliable data regarding the human health effects associated with exposure to DBPs. The primary drawback to attaining good epidemiologic data is the inability to accurately determine exposure (Weisel et al., 1999, Backer et al., 2000). In addition, the long latency period between exposure and health outcome (primarily cancers) makes it difficult to associate exposure with disease. There is a long period of time that must be examined in order to get an accurate measure of true exposure. The feasibility of attaining reliable exposure data over such long time expanses is severely limited. These are the problems that must be addressed in order to collect data that will prove beneficial in determining the true human health impacts of DBP exposure.

In the case of studying DBP exposure and cancer development, the latency period can extend over 30 years. King and Marrett (1996) found that people exposed to THM levels ≥50 μg/L for 35 years or more had 1.63 times the risk of bladder cancer compared to those exposed for less than 10 years (Backer et al., 2000). Furthermore,

Booker (2000) notes that a study performed by Cantor et al. (1987) reported that people who drank 8 cups/day of chlorinated tap water for 40-59 years had a 40% greater risk of bladder cancer than those who drank less tap water or unchlorinated water. In addition, people who drank at least 8 cups/day of chlorinated tap water for over 60 years had an 80% greater risk of developing bladder cancer. Trying to establish accurate exposure histories over such long time frames is highly unrealistic. There are far too many factors that can change over such long time frames that it is near impossible to obtain accurate records of study subjects or their water systems.

The primary problem with epidemiologic studies, as mentioned earlier, is determining an accurate measure of exposure. Nieuwenhuijsen et al. (2000) notes that exposure classification in the epidemiologic studies performed fall into three distinct groups. The first of these used water source and treatment as their exposure index (Aschengrau et al., 1993; Kanitz et al., 1996; Magnus et al., 1999). These studies did not look at measured DBP levels or any information on personal exposure characteristics, the exposure was simply assigned based on whether an individual lived in an area with specific water source and treatment conditions. This measure of exposure leads to misclassification of exposure, reducing precision and reliability of the studies (Weisel et al., 1999). One major problem with this exposure classification is that the variability in personal activities may lead to measurement error, and therefore attenuation of health risk estimates (Armstrong, 1998). In addition, Krasner (1999) notes that the composition of DBPs can differ depending on the total organic

carbon (TOC) and bromide levels, pH , temperature, fulvic or humic acid content and residual time allowed to react. As mentioned earlier, the animal toxicity and health effects vary among the DBPs. Different water sources will contain different concentrations of the various DBPs, therefore potential health effects may also vary. Epidemiologic studies that try to establish an association between exposure to a given compound and human disease are hindered by the uncertainty in DBP exposure. Variation in specific DBP concentration may play a role in potential health effects, therefore it is difficult to assign a health effect to exposure, since we are not sure what the individuals are exposed to. Krasner (1999) suggested that the "variation in findings in recent studies in California (Waller et al., 1998), North Carolina (Savitz et al., 1995) and Canada (Dodds et al., 1999) may be a result of the different composition of the DBPs in water".

Nieuwenhuijsen et al. (2000) mentions that the second class of exposure indices look at routinely collected DBP levels or models based on routinely collected levels (Kramer et al., 1992; Bove et al., 1995; Gallagher et al., 1998; Dodds et al., 1999). These measures also avoided including any information regarding personal exposure characteristics. The problems regarding variability in personal activities are significant in these exposure measures as well. The final exposure classification uses routinely collected DBP levels in conjunction with personal exposure characteristics such as ingestion, showering/bathing, and swimming (Nieuwenhuijsen et al., 2000). While this final group gives the most complete estimates of exposure, there are still

problems that affect the reliability of exposure data. Nieuwenhuijsen et al. (2000) notes that most studies do not obtain enough repeat measurements to give a good indicator of exposure. There are still unanswered questions regarding within- and between-subject variance, as well as difference in exposure based on variation in human activity. In addition, studies that rely on exposure questionnaires are subject to a good deal of uncertainty based on errors made by the study subjects filling in the questionnaires. Weisel et al. (1999) note that the variation in amount of water consumed, source of water consumed (tap, filtered, or bottled water may be drunk or used in preparing beverages or food), and frequency and duration of bathing and showering all have impacts on exposure. Exposure records must be accurate in order to be valid since the exposure to DBPs is multiroute. The different routes of exposure (such as ingestion, inhalation, and dermal contact) are very important, since the variability in the physicochemical properties of each DBP, the route of exposure, and activity patterns of the individual will affect true exposure to a given DBP (Wallace, 1997).

1.3.2.2 Biomarker research

The understanding that we do not have adequate individual measures of exposure to DBPs (Reif et al., 1996; Swan et al., 1998; Nieuwenhuijsen et al., 2000) provides the motivation for investigating biomarkers to DBP exposure. A biomarker of exposure is, in essence, a compound that is detected from biological samples that reflects exposure to a compound of interest. The advantage of using biomarkers is that the detected compound reflects what is actually in the body, rather than creating assumptions based on concentrations of DBPs in water and ingestion rates. Froese et al. (2001) suggest that a reliable biomarker of exposure to DBPs will enhance epidemiologic studies. To date, very little research has been performed to identify effective biomarkers of exposure to DBPs. Weisel et al. (1999) assessed both THMs and HAAs in the search for an effective biomarker of exposure. In the case of THMs, post shower breath samples correlated well with water concentrations, but background breath samples were essentially non-detectable (Weisel et al., 1999). Additionally, detectable levels of THMs in breath samples are only present for short periods of time (Weisel et al., 1999). Froese et al. (2001) suggest that the short duration of detectable THMs makes these compounds a poor biomarker of exposure. This is attributed to the rapid metabolism of THMs by the liver, particularly for ingested THMs, which are largely eliminated on the first pass of portal circulation from the gut to the liver. The investigation of urinary HAAs showed poor correlation between DCAA exposure and DCAA excreted in urine, because of rapid and variable

metabolism of this compound. TCAA, on the other hand, showed higher excretion levels for higher TCAA exposure versus low TCAA exposure (Kim et al., 1999; Weisel et al., 1999). Kim et al. (1999) found that by applying the amount of water consumed, the proportion of heated water used (estimating a 39% reduction in TCAA from boiling), and the use of home water filters (estimating a 70% reduction in TCAA from any filter type), a substantial correlation was found between estimated TCAA ingestion and measured excretion rate ($R = 0.73$, $n=42$). These data suggested that TCAA is a promising biomarker of exposure to DBPs.

Reif et al. (1996) note that "epidemiologic understanding of exposure-disease relationships generally evolves through an iterative process in which successive studies attempt to extend and improve upon earlier reports". With this in mind, the investigations I have performed with HAAs in beverages will serve to improve upon the previous HAA exposure data available. A better understanding of the fate of HAAs during beverage preparation will enhance our understanding of what is entering during ingestion. This information will enhance our ability to use HAAs as a biomarker of exposure, since we will know what and how much HAAs are entering the body through beverages. In addition, understanding the HAA profile in beverages will help us to estimate overall HAA exposure from measuring one compound (such as TCAA). Studies performed to examine the fate of THMs during beverage preparation have shown a decrease in their concentrations. Researchers have found that THMs decrease during boiling of water (Kuo et al., 1997; Kim et al., 1999), and

this discovery has been applied to beverages made with boiled water (such as coffee and tea). In order to obtain an accurate assessment of an individual's DBP exposure profile, it is important to understand exactly what and how much of each DBP is getting introduced into the body. These profiles have been shown to change during beverage preparation. Overall DBP exposure assessment will be affected based on the form in which water is ingested. In addition, enhancing the information regarding the use of TCAA as a biomarker will be affected. If we want to use TCAA as an indicator of exposure to DBPs in general, it is imperative that we understand how TCAA concentrations correlate with concentrations of other DBPs (in water and beverages).

1.3.3 Analysis of HAAs

Identification and quantification of DBPs from water samples is based on the extraction of the DBPs from a sample followed by identification using chromatographic methods. Detection of haloacetic acids in water samples is readily carried out in order to monitor public water supplies to ensure that they do not exceed the USEPA guideline of 60 μg/L for HAA5. The common method used is termed EPA Method 552 (Hodgeson et al., 1990), which involves liquid – liquid extraction of HAAs from drinking water, methylation by diazomethane, and analysis by gas chromatography with electron capture detection. While this method remains useful for the analysis of HAA5, the methodology was revised (USEPA Method 552.2) in

order to include the remaining three HAAs, which may be subject to future regulation (Magnuson and Kelty, 2000). This method, while quite similar to EPA 552, uses acidic methanol for methylation of the extracted HAAs, allowing identification of bromodichloro-, dibromochloro-, and tribromoacetic acid to give HAA9 (Munch et al., 1995).

While EPA Method 552 and 552.2 are effective in the identification and quantification of HAAs in water samples, a number of drawbacks are inherent to them. Extraction of the nonvolatile acids from water samples requires the use of methyl *tert* butyl ether (MTBE) as an organic phase. The initial noted drawback to the commonly used methods is the fact that MTBE is toxic and a suspect carcinogen (Martinez et al., 1998; Ells et al., 2000). In addition, the use of diazomethane to derivitize the nonvolatile acids in Method 552 increases health concern, as this compound is highly inflammable and carcinogenic (Martinez et al., 1998; Ells et al., 2000). Beyond the health concerns associated with these methods, they are also time and labor intensive (Martinez et al., 1998; Ells et al., 2000; Sarrion et al., 2000). Magnuson and Kelty (2000) also note that the additional three HAAs detected by EPA Method 552.2 (bromodichloro-, dibromochloro-, and tribromoacetic acid) are subject to decarboxylation during methylation, which affects the reliability of detection. The reliability of EPA Method 552.2 is very matrix and operator dependent (Magnuson and Kelty, 2000). The aforementioned problems have given rise to the development of new methods for the analysis of HAAs in water samples.

Measures have been taken to minimize contact with toxic substances, reduce the time and labor expended per sample, and increase the sensitivity and reliability of the analysis.

In order to minimize exposure to toxic compounds during extraction of the HAAs, some individuals have turned to solid phase extraction (SPE) rather than liquid-liquid extraction (LLE) (Martinez et al., 1998; Magnuson et al., 2000). This method utilitizes a sorbent fixed to a solid support that binds the HAAs as they contact the sorbents. In doing this, HAAs are extracted and then eluted using a methanol-water solution. These samples are then derivitized and analyzed, usually by GC-ECD. This methodology reduces operator contact with toxic solvents (Martinez et al., 1998). In addition, errors introduced by the operator are expected to be reduced, since the operator is not physically performing a solvent extraction (as is done in LLE).

Solid phase microextraction (SPME) is another emerging extraction technique being used to avoid some of the drawbacks associated with LLE (Pawliszyn, 1997). SPME is a solvent free method for extracting organic compounds from solid and liquid samples (Dugay et al., 1998; Sarrion et al., 1998; Sarrion et al., 1999). The basis of SPME involves fused-silica fibers that are coated with a polymer that binds target analytes. Analytes can be extracted from gaseous or liquid media and are bound to the SPME fiber. "After equilibrium is reached or after a well-defined extraction time, the compounds absorbed are thermally desorbed by exposing the fiber in the injection

port of a gas chromatograph, or redissolved in an organic solvent if coupled to HPLC" (Sarrion et al., 1999). Therefore, sample extraction, concentration, and introduction for analysis are integrated into a single step (Sarrion et al., 1999). The advantages of this extraction technique lie in the fact that there is minimal exposure to toxic chemicals used in LLE, the method is rapid (due to the integration of a number of steps), and operator error is minimized by eliminating an extraction step. In addition, the ability to sample from a gaseous medium allows the analysis of headspace samples. This results in the ability to sample HAAs from much more complex matrices than water alone, because interference from the complex matrix is avoided.

While this method is efficient in terms of time and labor, there are a few drawbacks that should be noted. Since this is a relatively new procedure, there has not been a thoroughly validated analytical method using SPME for the analysis of HAAs in water. As a result, there are still uncertainties in precision and accuracy when using SPME for HAA analysis. Furthermore, differences in the polymer coating, extraction time, and extraction and desorption temperatures will affect the detection of HAAs. Sarrion et al. (1999, 2000) have noted that HAA analysis can be optimized for each particular acetic acid, therefore a single analytical method may not be optimal for the detection of all HAAs. Finally, Sarrion et al. (1999) showed that the sample matrix can interfere with the extraction of the HAAs. These drawbacks should be taken in to account when using this method. In my research, we are comparing TCAA and

DCAA concentrations rather than looking at a large number of DBPs, therefore choosing one polymer coating and analytical method is acceptable. A larger concern for my work lies in the uncertainty with regard to matrix effects. The beverage matrix may affect the SPME fiber differently than the water matrix, which can affect the observed results. Beverages may contribute a large number of compounds to the headspace which can compete with TCAA and DCAA for adsorption sites on the SPME fiber.

Investigations of HAAs in water samples have also been carried out using methods much different than EPA Methods 552.0 and 552.2. Magnuson and Kelty (2000) used flow injection analysis coupled with electrospray mass spectrometry to analyze HAA9 from water samples. This method minimizes the use of MTBE and does not require derivitization of the extracted acids, which prevents the decomposition of the HAAs (Magnuson and Kelty, 2000). This alternative method reportedly performed well statistically when compared to HAA6 analysis done via EPA Method 552.2 (Magnuson and Kelty, 2000).

A final analytical method currently being assessed is the combination of electrospray ionization, high-field asymmetric waveform ion mobility spectrometry, and mass spectrometry (ESI-FAIMS-MS) (Ells et al., 1999; Ells et al., 2000). This method is highly desirable in that no extraction or derivitization steps are needed. The HAAs are directly analyzed from the water samples. Mason and McDaniel (1988) reported

that there are compound dependent differences in ion mobility between low and high electric fields. Therefore, separation of ions is based on their mobility in a high electric field (Ells et al., 2000). This is the basis of ion separation by the FAIMS analyzer. After electrospray ionization, ions are separated based on their mobility in a high electric field, and analyzed by MS. This method reports detection limits near those reported for EPA Method 552.2 (< 0.5 μg/L) for most HAAs and substantially improved detection limits for tri-halogenated HAAs, and extremely fast run times (approximately five minutes per sample) (Ells et al., 2000).

With the advent of new analytical procedures for HAA detection, our understanding of the fate and behaviour of HAAs in the environment stands to increase significantly. In the case of our research, the advent of SPME allows us to analyze complex matrices (various beverages) without interference from the matrix. Derivitization of the HAAs in the beverage samples volatilizes the HAAs and allows us to selectively extract the HAAs from the sample using an SPME fiber.

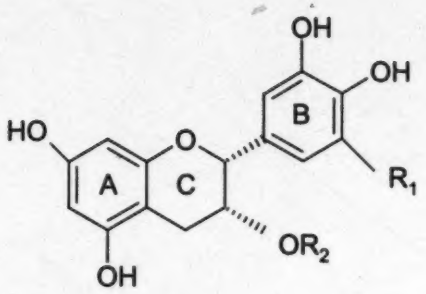
1.4 Beverage Research

1.4.1 Why Analyze DBPs in Beverages?

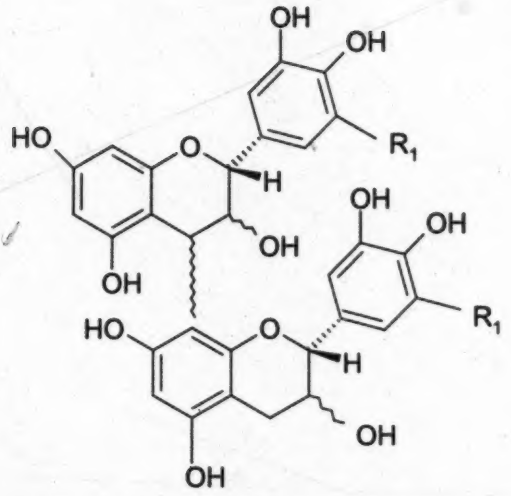
Investigating the fate of HAAs in beverages is justified because exposure data is very limited. We want to enhance our knowledge of human exposure by gaining a comprehensive understanding of exposure. In addition, recent investigations into

TCAA as a biomarker of exposure will be improved, as we cannot account for all ingestion sources of TCAA (and the other DBPs) through tap water alone. Raymer et al. (2000) notes that consumption surveys (EPA, 1997) indicate that approximately 2/3 of the drinking water ingested is through other sources, such as frozen juices, coffee, tea, etc. The predominant route of exposure to the relatively nonvolatile HAAs is ingestion (Kim et al., 1999; Weisel et al., 1999; Nieuwenhuijsen et al., 2000). The only way to obtain an accurate estimate of exposure to HAAs is to have a good understanding of HAA levels in the different forms of water consumption. Nieuwenhuijsen et al. (2000) notes that "these nuances in the form the water is consumed can make a considerable difference to exposure levels". With this in mind and the fact that little work has been performed to characterize the levels of HAAs in beverages, it is important to gain an understanding of the fate of HAAs when beverages are prepared. Observed differences in HAA levels in beverages compared to water samples will have an impact on exposure levels, since the bulk of water ingestion is through other beverages. In addition, all epidemiologic studies have focused on HAA exposure from tap water samples.

Formation of HAAs is based on the interaction of chlorine with natural organic matter in the water supply. Treated water contains a residual amount of chlorine to destroy any microbial pathogens that might be introduced to the water supply while it makes its way to consumer's homes. The residual chlorine has the capacity to react with any



Structure of catechins



Structure of flavanols

Figure 1.2.: The basic chemical structures of catechins and flavanols, both of which are components of tea and coffee.

organic matter that may be introduced to the water supply in the distribution system. Most beverages are a mix of sugars and other organic compounds. It is feasible that the residual chlorine may react with the organic load introduced by a beverage to form additional DBPs, including TCAA and DCAA. It is this hypothesis that necessitated the investigation into the fate of HAAs during beverage preparation. Analysis of a number of beverages compared to the water samples used to make them will give us an indication of the fate of HAAs during beverage preparation. Hopefully these data will paint a clearer picture of HAA exposure from water ingestion.

1.4.2 Rationale for formation of DBPs from organic carbon in beverages

Traditionally, formation of DBPs has been attributed to halogen substitution reactions or oxidation reactions that occur between disinfectants used and natural organic matter (NOM) found in water (Krasner, 1999). Krasner (1999) notes that the USEPA has determined 196 compounds whose formation could be attributed to the chlorination process. It is clear that there is an immense potential for reactions between chlorine and organics found in drinking water. The introduction of various organic components from beverages increases the possibility of the aforementioned reactions to occur because of the increased amount of organics in the water. Norwood et al. (1987) suggest that there are a myriad of aliphatic and aromatic structures that can lead to organic halogen formation. The electrophilic nature of

aqueous chlorine species makes them highly reactive with electron rich sites in organic structures (Rook, 1977; Norwood et al., 1987; Reckhow and Singer, 1985). Harrington et al. (1996) suggest that activated aromatic rings, aliphatic β-dicarbonyls, and amino nitrogen are examples of electron rich organic structures that can react strongly with chlorine.

Chemical analysis of beverages has provided insight into some of the chemical compounds present once a beverage is prepared. Yang and Landau (2000) note that green tea contains a large number of polyphenolic compounds, of which most are catechins. They suggest that a "typical tea beverage, prepared in a proportion of 1g leaf to 100mL water in a 3-min brew, usually contains 250-350 mg tea solids, comprised of 30-42% catechins and 3-6% caffeine. A generalized structure of catechins is shown in Figure 1.2. Norwood, Christman, and Hatcher (1987) note that phenolic ring rupture mechanisms mediated by aqueous chlorine are very important in the formation of organic halogens. This hypothesis was derived from the haloform reaction (Figure 1.3), proposed by Rook (1977). With such a substantial amount of phenolic compounds able to react with residual chlorine, it is reasonable to assume that additional HAAs will form during beverage preparation. In addition, caffeine is also a ring structure that can be attacked by the chlorine molecules, possibly forming DBPs. Lancashire (2001) notes the immense number of compounds that are flavor constituents of roasted coffee. Table 1.2 lists the different compounds and the number of different varieties of each. In addition, Figure 1.4 illustrates the chemical

structures of a number of the organic compounds found in coffee that have the potential for reaction with chlorine to form DBPs.

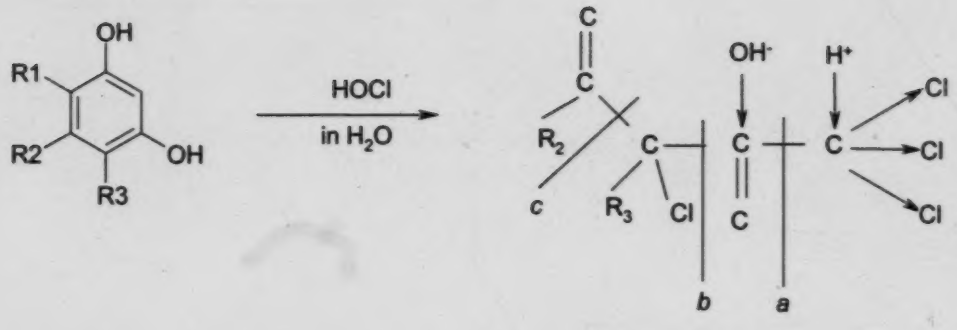


Figure 1.3: The haloform reaction as suggested by Rook (1977). This reaction is the general foundation for the formation of DBPs in drinking water. The pathway involves a fast chlorination of the carbon atoms that are activated by ortho hydroxide (OH) substituents or phenoxide ions in an alkaline environment. After the aromatic structure has been halogenated and opened, cleavage at *a* will result in the formation of THMs (e.g. chloroform [$CHCl_3$]). Oxidative and hydrolytic cleavage at *b* will result in HAA formation (e.g. trichlororacetic acid [Cl_3CCOOH]) or chloral hydrate ($Cl_3CCH(OH)_2$). Finally, cleavage at *c* will result in haloketone formation. The presence of bromide will result in the formation of mixed bromochloro by-products. Explanation of the reaction was taken from Krasner (1999).

TYPE	NUMBER
furans	99
pyrazines	79
ketones	70
pyrroles	67
hydrocarbons	50
phenols	42
esters	29
aldehydes	28
thiazoles	28
oxazoles	27
thiophenes	26
amines + N-containing	24
alcohols	20
acids	20
sulfides + S-containing	16
pyridines	13
not classified	9
lactones	8
TOTAL	**655**

Table 1.2: Summary of flavor constituents found in roasted coffee aroma (Lancashire, 2001).

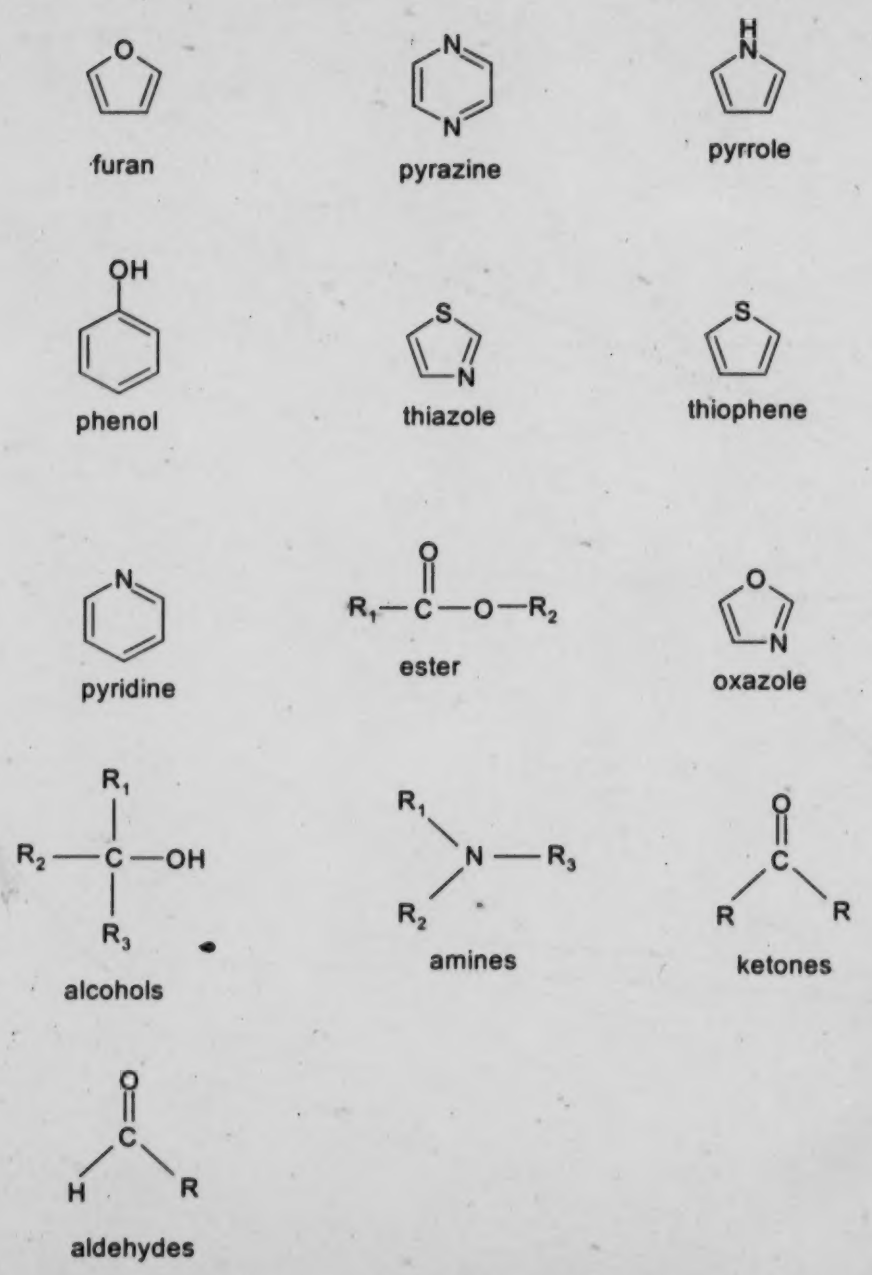


Figure 1.4.: Structures of some of the organic constituents found in coffee that are available to react with residual chlorine found in the water.

Pascual-Teresa et al. (1998) note that beverage composition consists of flavanols, which consist of a large variety of polyphenolic compounds. This again provides a large number of organic compounds that are available to react with chlorine. Flavanols may vary in composition and amount among beverages, but it appears that their presence is ubiquitous among all beverages. Therefore it is plausible to assume that all beverages prepared have the potential for excess DBP formation. Given the fact that electron rich sites in organic molecules are highly subject to reactions with chlorine (Rook, 1977; Norwood et al., 1980; Reckhow and Singer, 1985), the numerous polyphenolic compounds introduced by coffee, tea, and other beverages provide high potential for formation of DBPs. Figure 1.2. illustrates some of the polyphenolic compounds (catechins and flavanols) provided by beverage substrates.

The presence of organic compounds in coffee such as 2,3- pentanedione (Roberts et al., 2000) further supports the hypothesis that organic compounds introduced by a beverage can provide precursors for the formation of DBPs. The above mentioned compound is a diketone, which are another prime reactant involved in the haloform reaction proposed by Rook (1977). Krasner (1999) notes the observation of Morris and Baum (1978) that the halogenation and hydrolysis reactions characteristic to the haloform reaction typically occur with methyl ketones or compounds oxidizable to that structure. Furthermore, Morris and Baum (1978) noted that β-diketones are a group of compounds that can readily react with chlorine to form DBPs. In addition, these compounds can react relatively quickly to form DBPs. The diketone structures

present after coffee brewing suggest that there are additional compounds present that are known to readily react with chlorine to form DBPs. This theory is facilitated by Reckhow and Singer (1987), who suggest that β-diketone moieties are paramount in the formation of DBPs.

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Chapter 2: Chemical Analysis of Water and Beverage Samples

2.1 Introduction

Accurate assessment of human exposure to DBPs relies on the ability to establish a valid measure of how much of these compounds are available to be introduced to a human being. TCAA and DCAA are introduced primarily through ingestion of drinking water (Kim et al., 1999; Weisel et al., 1999). Based on this knowledge, epidemiological assessment of human exposure has been determined through the analysis of drinking water supplies and amount of water ingestion. However, on average approximately 2/3 of all drinking water consumption is through sources that are not pure tap water, such as beverages prepared with tap water (coffee, tea, juice mixes) and commercially prepared (bottled) water and beverages (EPA, 1997). In addition, the National Human Activity Pattern Survey (NHAPS) presented in Robinson and Blair (1995) notes that 61% of respondents ingested juice mix on a daily basis. This survey was based on 24 hour time/activity diaries compiled by 9386 people residing in the continental U.S. from 1992 to 1994. With this in mind, it is clear that a true estimate of exposure needs to take in to account the TCAA and DCAA contribution via these other beverages. Accurate exposure analysis rests on the ability to gain a comprehensive understanding of all exposure routes (Nieuwenhuijsen et al., 2000; Raymer et al., 2000; Froese et al., 2002). This general

foundation of knowledge facilitated the need for assessing the fate of TCAA and DCAA during beverage preparation.

Very little research has been performed to assess exposure to HAAs from beverages. Presence of DBPs (such as THMs) in beverages have been indicated by reports released by the US Food and Drug Administration (FDA) (Heikes et al., 1995; McNeal et al., 1995). More recently, Raymer et al. (2000) have developed a method to detect the presence of a variety of DBPs (including HAAs) in foods and beverages. While work has been done to identify the presence of DBPs in beverages, there is no evidence to indicate the fate of HAAs in tap water when beverages are prepared. This question can only be answered by quantitatively examining TCAA and DCAA concentrations in tap water and beverage samples. Comparison of concentrations between beverages and the water used to prepare them will provide an indication of the fate of these compounds after the beverage preparation process.

2.2 Scope

The objectives of the work presented in this chapter were threefold. First, we wanted to determine the concentration of TCAA and DCAA in water samples and beverage samples made with the same water. Second, the fate of TCAA and DCAA during production was examined by comparing the levels of these chemicals in water and beverages made with the water. Finally, work was performed to identify the

relationship between residual chlorine concentration and TCAA/DCAA production in beverages. It is known that the formation of DBPs is dependent upon the reaction of chlorine or other oxidizing agents with organics in water. Introducing an organic load such as coffee grounds, tea leaves, or iced tea crystals generates the potential for DBP formation by the reaction with residual chlorine in the water. This analysis serves to illustrate the fact that these reactions do occur when beverages are prepared.

2.3 Materials and Methods

2.3.1 Sample Preparation

2.3.1.1 Beverage Preparation

Beverages were prepared in a consistent and controlled manner that replicated everyday preparation methods. Coffee samples were prepared in a common drip coffeemaker (Mr. Coffee Inc.; *International* Series, Bedford Heights, OH). In order to minimize variation, only one brand of coffee grounds (Maxwell House Original) were used throughout the study. 500mL of water were added to the coffeemaker and one standardized scoop (approximately 8.6g) of coffee was used. In addition, only one brand of coffee filter was used. These filters were made with unbleached paper and water samples processed through the coffeemaker (without coffee grounds added) were analyzed to ensure that the filters did not contribute to HAA concentrations. Upon completion of brewing, 40mL aliquots were placed in 50mL polycarbonate centrifuge tubes (Corning Incorporated, Corning, NY) and allowed 20 minutes to cool

to room temperature (approximately 23^0C) to prevent a violent exothermic reaction when the samples are acidified with sulfuric acid. Analysis was then carried out on the samples (Section 2.3.3).

Tea samples were prepared using one bag of generic Earl Grey tea and 500mL of water boiled in an electric kettle for five minutes. Samples were stirred for two minutes and then aliquotted into 50mL polycarbonate centrifuge tubes (as done with the coffee samples). Tea samples were also allowed to cool for 20 minutes.

Iced tea samples were prepared using 500mL of cold water and one standardized scoop of iced tea crystals (approximately 8.6g). Upon addition of the crystals, the samples were stirred for two minutes. Once mixing was complete, 40mL aliquots were placed into 50mL polycarbonate centrifuge tubes.

2.3.1.2 Creating a Range of Residual Chlorine Concentrations

In experiments to determine the effect of residual chlorine concentration on HAA formation, samples of Milli-Q water were spiked with a free chlorine source. Milli-Q water is tap water that has been purified by reverse osmosis followed by several filtration steps and UV irradiation. It contains very low levels of residual chlorine and organics, and is used as a lab standard for "pure" water. The chlorine source used was Javex bleach (Colgate-Palmolive, Toronto, Canada) that is given as 5.25% available chlorine, which is chlorine in a form that is free to react with other compounds, such as HOCl. In order to identify any major matrix effects that could jeopardize the experiments, Milli-Q water was spiked to a chlorine residual concentration similar to that of Edmonton tap water. Headspace samples of spikes and tap water were analyzed by GC-ECD (Section 2.3.3). Following this, the samples were compared to determine any large discrepancies in compounds that were detected. At the concentrations used, there were relatively small differences in the baselines on chromatograms of tap water and the spiked water, indicating little to no matrix effects due to the added chlorine. Table 2.1 indicates the TCAA and DCAA concentrations detected in Milli-Q water, Milli-Q water spiked to a residual chlorine concentration close to that of tap water, and tap water samples (Sample analysis methodology in Section 2.3.3.2). The TCAA concentration increased by a factor of 1.5 and DCAA concentration increased by a factor of 4 when the Milli-Q water was spiked. Also, the Milli-Q spike had a residual chlorine concentration close to that of

the tap water, and the level of TCAA was approximately 10 fold lower, while DCAA was approximately 1.4 fold lower than tap water. This provided some evidence that the spiking with chlorine bleach was not substantially altering the levels of the analytes in question. In addition to this, Figures 2.1a through 2.1c are the chromatograms obtained from each of these analyses. Table 2.1 indicates the TCAA and DCAA concentrations detected in the three different water samples. Spiking the water samples did not alter the chromatographic profile to a degree that would cause concern. With this evidence, it appeared that spiking with bleach proved to be a viable option for creating a range of residual chlorine concentrations for experimentation. In addition, the spike samples were analyzed for *free chlorine* (HOCl), therefore the concentrations detected indicate the amount of free chlorine available to react with the beverages. Furthermore, Lambusch (1971) notes that household bleach has 3-5% available chlorine, which is consistent with the free chlorine percentage indicated on the bleach container. This reduces concern with regard to additional forms of chlorine in the commercial bleach that are free to react. The mechanical aspect of spiking involved direct inoculation of Milli-Q water with a volume of Javex bleach using an Eppendorff micropipetter. A stock solution of spike was prepared, followed by residual chlorine analysis (Section 2.3.2) to determine the residual chlorine concentration. Once a suitable stock was prepared, dilutions were made to establish a range of residual chlorine concentrations to be used in the preparation of beverages.

While there is reasonable evidence suggesting that Javex did not significantly interfere with the matrix being studied, it should be noted that that chlorine residual in spiked water is a free chlorine source (HOCl), while tap water contains chloramine. The spiked water samples were used to identify differences in HAA formation as the residual increased. Since there was no cross-comparison between tap water and spiked water samples, there should not be any concerns with using a different chlorine source for the residual chlorine analysis. Samples were analyzed to see the difference in HAA levels relative to each increasing residual chlorine concentration. Since all samples were prepared the same way, this comparison should provide reliable results for the purpose intended. The intent of this experiment is to show a *relationship* between residual chlorine concentration and HAA concentration in beverages.

Sample	[TCAA] (μg/L)	[DCAA] (μg/L)
Milli-Q ($[Cl_2]_{free}$= 0.03mg/L)	0.35	0.51
Spiked Milli-Q ($[Cl_2]_{free}$ = 2.19mg/L)	0.48	1.9
Tap water ($[Cl_2]_{chloramine}$ = 2.15mg/L)	4.0	2.6

Table 2.1: TCAA and DCAA concentrations in three different water samples. Full sample data can be found in Appendix A: Tap water: Table A1; Milli-Q: Table A4; Spiked Milli-Q: Table A8.

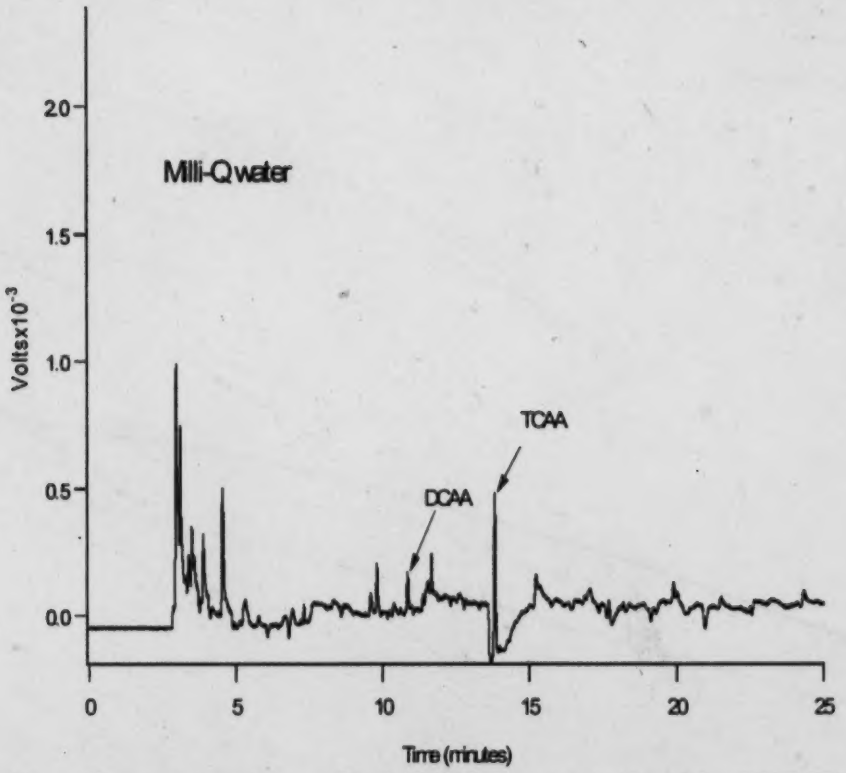

Figure 2.1a: Chromatographic profile for Milli-Q water analyzed by GC-ECD.

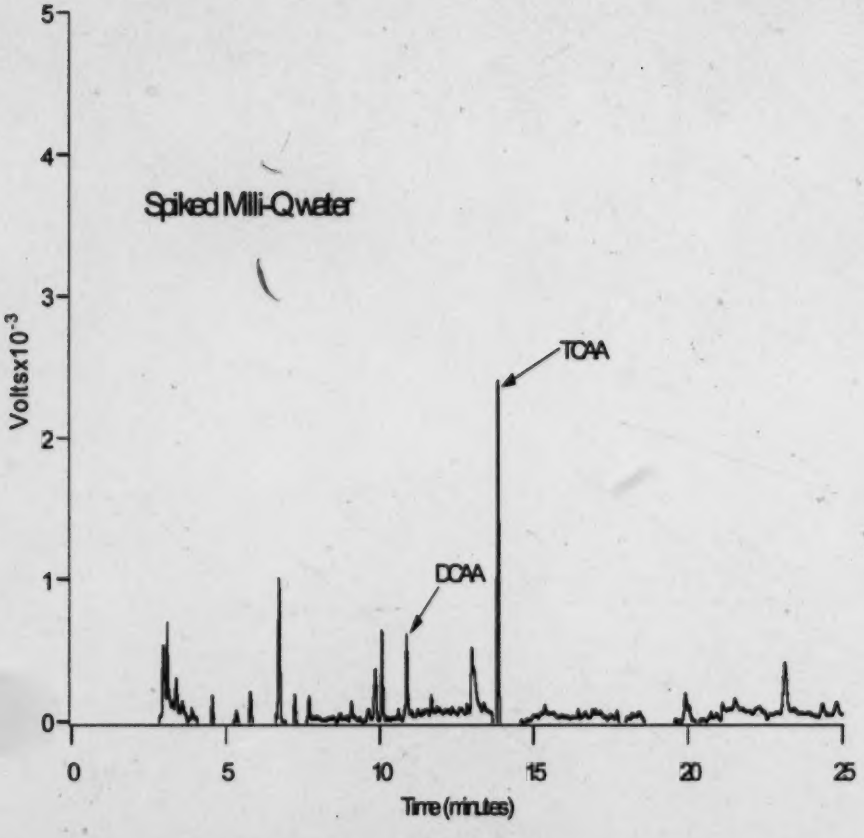

Figure 2.1b: Chromatographic profile of spiked Milli-Q water analyzed by GC-ECD. The Milli-Q water was spiked with Javex bleach (5.25% available chlorine) to a final chlorine residual of 2.19mg/L.

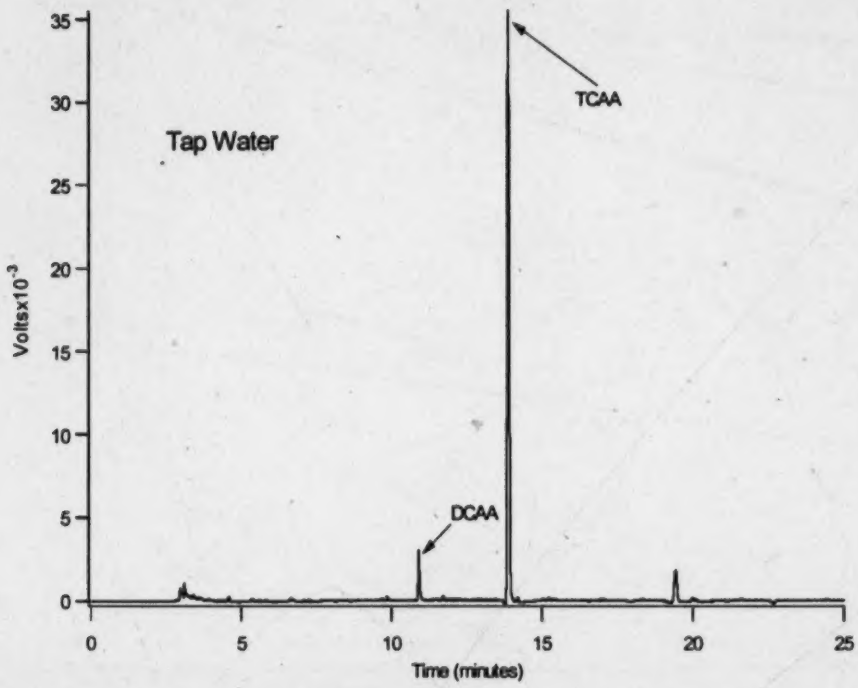

Figure 2.1c: Chromatographic profile of tap water analyzed by GC-ECD.

2.3.2 Residual Chlorine Analysis

All water was tested for residual chlorine concentrations prior to beverage preparation. Residual chlorine testing was carried out using a hand held chlorine detector (Hanna Instruments Inc.; model HI 93734, Woonsocket, Rhode Island). The detector kit analyzes for free and total chlorine with a range of 0.01 mg/L to 10.00 mg/L. The analysis is an adaptation of the EPA recommended *N,N*-diethyl-*p*-phenylenediamine (DPD) method 330.5. Chlorine in the water reacts with the DPD reagent to produce a colored product that is detected by the instrument (which is essentially a hand-held spectrophotometer). Intensity of the color will affect light absorbance, and the level of light absorbance is relative to a specific chlorine concentration. Accuracy and precision of the instrument was tested by statistical analysis of triplicate tests in order to identify variance from one sample to the next. The method detection limit data and comparisons of our acquired data with that from residual chlorine analysis from Edmonton's water treatment plants are presented in Appendix A. The MDL was found to be 0.06mg/L, therefore values below this (which was often the case for Milli-Q water) cannot be deemed reliable.

Residual chlorine analysis of Edmonton tap water was carried out for total chlorine rather than strictly free chlorine. This was done due to the method of water treatment implemented by the municipal water service. Water is taken from the North Saskatchewan river and receives conventional treatment consisting of alum

flocculation and rapid sand filtration with free chlorine added after clarification, but before filtration. Ammonia is then added to form chloramines prior to storage in reservoirs (to achieve adequate contact time with the chlorine). Based on water quality data from 1996 – 1998, the target total chlorine residual tends to sit at an average of approximately 2.5mg/L. Table A14 (Appendix A) illustrates a comparison between residual chlorine concentration detected in our tap water samples and those detected at Rossdale water treatment plant (our water source) by EPCOR technologists. The lower values observed by our detector are expected, since there is generally some degradation of residual chlorine during the time that the water makes its trip from the treatment plant to our tap. It should be noted that the decrease is fairly stable, and the variance in values, both for our data and EPCOR's, is rather minimal.

Spiked Milli-Q samples (described in Section *2.3.1.2*) contained a free chlorine source, therefore they were tested for free chlorine. As mentioned earlier (*2.3.1.2.*), there are inherent differences between free chlorine (HOCl) and chloramines (NH_2Cl, $NHCl_2$, NCl_3). Speitel (1999) notes that formation of DBPs can still be expected with chloraminated water sources, but at lower concentrations than that of free chlorine, due to the weaker oxidizing power of chloramines. Speitel (1999) mentions that chloraminated water always contains some free chlorine, therefore similar reactions are expected to occur.

2.3.3 Chemical Analysis of Water and Beverage Samples

2.3.3.1 Sample Extraction and Derivitization

The 40mL sample aliquots (held in 50mL polycarbonate centrifuge tubes) were extracted using 2mL of concentrated H_2SO_4 (to adjust the pH to approximately 0.5). Following this step, 12g of Na_2SO_4 was added to saturate the sample with salt. At this point, 4mL of methyl-*tert*-butyl ether (MTBE) was added to create an organic layer that held the HAAs of interest (namely TCAA and DCAA). Upon addition of these reagents, samples were shaken vigorously for 9 minutes on a table top shaker (Janke and Kunkel; model VX8) in order to ensure effective mixing of all chemical constituents. Samples were then centrifuged at 3000 rpm for 8 minutes to remove most of the organic components from the organic layer. Following centrifugation, the organic layer was removed using a glass micropippeter and placed in 10mL crimp top SPME vials. In order to concentrate the TCAA and DCAA in the sample, the solvent was evaporated at 50^0C under a stream of pure nitrogen gas on a heating block (Pierce Reacti-Therm). The nonvolatile nature of TCAA and DCAA ensured that we would not lose these compounds during the evaporative step. Post evaporation, the HAAs were derivitized by addition of 100μL of methanol and 50μL of concentrated H_2SO_4. Samples were capped with crimp tops and allowed to sit on a heating block for 10 minutes at 50^0C to allow for complete derivitization. This method of extraction and derivitization was used for all samples analyzed.

2.3.3.2 Sample Analysis Using GC-ECD

All samples were analyzed by SPME headspace sampling followed by gas chromatography with electron capture detection (GC-ECD). Headspace samples were collected on SPME fibers (100μm, polydimethylsiloxane) that were supplied by Supelco. Automated sampling of the headspace in the 10mL vials involved inserting the SPME needle into the septum of the 10mL vials and allowing adsorption of the analytes on to the SPME fiber for 10 minutes at room temperature (approximately 23^0C) using a Varian 8200 autosampler. Once the samples were taken, desorption of the derivitized HAAs took place in the GC injector. The injector was set for splitless injection at 250^0C, and sample desorption was allowed to take place for two minutes.

Following desorption, the HAAs were separated using a DB-5.625 capillary column (30m x 0.25mm i.d., 0.25μm film thickness; J&W Scientific) installed in a Varian CP-3800 gas chromatograph. The GC-ECD analysis involves a temperature program starting at 40^0C (held for 5 minutes) followed by a temperature ramp of 5^0C/minute to 75^0C and held for 15 minutes; temperature was then ramped at 20^0C/minute to 150^0C and held for one minute. This temperature program proved sufficient for clear separation of the compounds of interest (namely TCAA and DCAA). Chromatographic data was obtained and processed using Varian Star software (version 5.3, Varian Chromatography Systems, Walnut Creek, Ca.). Concentrations

of each analyzed compound were determined using pre-run calibration curves (Section 2.3.5).

2.3.4 Method Detection Limit Studies

Samples of Milli-Q water were spiked with either pure TCAA at ≥99.5% purity or DCAA at ≥99% purity (supplied by Supelco) to a concentration of 0.1μg/L for TCAA and 1.0μg/L for DCAA. The different concentrations were used due to the fact that earlier research indicated greater sensitivity for TCAA compared to DCAA using the applied method (Mike Ongley, personal communication, 2000). Following spiking of samples, 40mL aliquots were extracted, derivitized, and analyzed as described above. In order to determine the MDLs, seven replicate samples of each compound were analyzed. The method detection limit (MDL), as defined in the U.S. Federal Code of Regulations, is a measure of the precision of replicate analyses of a sample (Magnuson and Kelty, 2000). Calculation of the MDL was carried out as described by Raymer et al. (2000) and Magnuson and Kelty (2000). The standard deviation from seven replicates was multiplied by 3.143 (student *t* value for one tailed distribution with 6 *df* at the 0.99 confidence level) in order to obtain the MDL (Glaser et al., 1981).

The MDL calculated for TCAA was 0.071 μg/L, a value much lower than the MDLs reported using other extraction and analysis methods. Raymer et al. (2000) reported

an MDL for TCAA in orange juice samples at 2.5 μg/L (using a modified liquid-liquid extraction method). This number is approximately 26 times greater than our value. The MDL reported by Magnuson and Kelty (2000) using electrospray-MS was 0.13 μg/L. The MDL for DCAA, while considerably greater than that for TCAA, was still acceptable. A value of 0.87μg/L was obtained for DCAA. This value was again lower than that obtained by Raymer et al. (2000), who obtained a value of 13μg/L. The value of 0.32μg/L reported by Magnuson and Kelty (2000) was somewhat lower than our value. Overall, the SPME method provided MDLs that were very low relative to those reported using other popular analytical procedures currently in practice. In addition, the average concentration of TCAA was found to be 0.11μg/L for the seven spiked samples used for the MDL analysis. The spike concentrations were 0.10μg/L, therefore the average concentration detected was very close to the actual amount, indicating that the accuracy of our method is acceptable. The average analyzed concentration of DCAA (spiked to 1.0μg/L) was 0.84μg/L, which is again quite close to the true value. These results, along with the MDL values, attest to the precision and accuracy of the employed method. All of the data for this experiment can be viewed in Appendix A, Tables 12a and 12b.

2.3.5 HAA Standards and Calibration

Separate stock solutions of TCAA and DCAA were prepared with Milli-Q water to construct calibration curves in order to quantify these two compounds in water and

beverage samples. The stock TCAA (≥99.5% purity, Supelco) and stock DCAA (≥99% purity, Supelco) were added to Milli-Q water in order to make a stock solution of known concentration. The stock prepared was approximately 1.0 mg/mL, and each stock was discarded after one week. The calibration series for both compounds generally consisted of 1, 5, 10, 25, and 50 µg/L. Calibrations would generally be run each week prior to sample analysis. These calibration curves enabled the operator to monitor the performance of the instrument and compensate for minor differences in the everyday detection of compounds. Construction of the calibration curves involved selecting the peak of interest manually and then integrating to determine the area under the peak. The calibration curves were then constructed using Varian Star software (version 5.3, Varian Chromatography Systems, Walnut Creek, Ca).

2.3.6 Statistical Analysis

The ability to evaluate the performance of the implemented method involved statistical analysis of results to establish standard deviations among replicate samples. The bulk of this statistical analysis involved the use of the basic statistics functions in Excel (Microsoft, 2000). Determination of standard deviations for MDLs also involved the use of Excel. Determining the effects of residual chlorine on the production of TCAA and DCAA in beverages involved the construction of graphs, and this was done using SigmaPlot 2000 (SPSS, 2000).

2.4 Results and Discussion

2.4.1 Analysis of Water and Beverages

2.4.1.1 Analysis of Beverages Prepared with Cold Tap Water

Chromatographic analysis of water and beverage samples clearly indicated greater concentrations of TCAA and DCAA in beverages compared to the water used to make them. This section indicates the concentrations of TCAA and DCAA reported in tap water and coffee, tea, and iced tea. The data was analyzed to identify relative change in TCAA and DCAA concentrations when beverages were prepared. Coffee prepared with cold tap water showed significant increases in TCAA and DCAA concentrations. In addition, tea samples and iced tea samples all indicated percentage increases in TCAA and DCAA when compared to the water used to prepare them. These results clearly indicated the production of TCAA and DCAA during beverage preparation. Table 2.2 indicates the TCAA and DCAA concentrations in tap water and coffee prepared with the water, as well as the relative increases observed for each compound.

	TCAA			DCAA		
	[Water] (μg/L)	[Coffee] (μg/L)	Relative Increase (%)	[Water] (μg/L)	[Coffee] (μg/L)	Relative Increase (%)
Experiment 1 [Cl] = 2.18mg/L	2.8 ± 0.9	7.7 ± 1.6	180	3.0 ± 0.8	36 ± 5.8	1100
Experiment 2 [Cl] = 2.30mg/L	5.3 ± 1.7	7.4 ± 0.7	40	8.3 ± 6.2	26 ± 1.8	220
Experiment 3 [Cl] = 2.23mg/L	3.5 ± 0.1	9.7 ± 1.0	180	3.6 ± 0.7	29 ± 2.4	720
Experiment 4 [Cl] = 2.27mg/L	3.5 ± 0.1	6.8 ± 1.0	94	3.0 ± 0.5	20 ± 3.7	580
Experiment 5 [Cl] = 2.22mg/L	4.8 ± 3.0	6.1 ± 1.1	27	4.9 ± 3.4	15 ± 2.3	210
Experiment 6 [Cl] = 2.21mg/L	2.7 ± 2.1	8.2 ± 0.6	200	3.6 ± 2.0	32 ± 2.6	780
Experiment 7 [Cl] = 2.15mg/L	4.8 ± 1.4	6.6 ± 0.3	38	2.8 ± 0.4	22 ± 2.4	700
Averages	**3.9 ± 1.3**	**7.5 ± 0.9**	**110 ±76**	**4.2 ± 2.0**	**26 ± 3.0**	**610 ± 320**

Table 2.2: The average TCAA and DCAA concentrations in triplicate samples of tap water and coffee. Residual chlorine levels in the tap water used and percent increases of TCAA and DCAA are also indicated.

Table 2.2 indicates the significant increases observed when coffee is prepared using cold tap water. Overall, the relative increase in TCAA concentration after coffee preparation is 110%, and the relative increase of DCAA is 610%. The large standard deviation is due to the significant variation between experiments. Since the concentrations of TCAA and DCAA are relatively low in both the water and beverage samples, even minor differences in concentration can significantly affect the relative increase observed. Experiments that showed higher variation in triplicate analyses of a sample resulted in giving a large range of relative increase. This means that samples showing considerably high or low relative increases (that ultimately affect the overall standard deviation of relative increase) may be a result of averaging the three separate analyses. As stated earlier, any outlier in TCAA or DCAA concentration will significantly affect the observed relative increase. In the case of coffee (Table 2.2), the variation in relative increase is too great to be able to accurately pinpoint a measure of relative increase. It can only be noted that the trend is consistently towards an increase in TCAA and DCAA during beverage production. The full data set is provided in Appendix A (Raw Data), Table 1.

Analysis of tea samples indicated similar trends with regard to the increase in TCAA and DCAA when the beverage was prepared. Table 2.3 shows the average TCAA and DCAA concentrations, as well as relative increases, in water samples and tea samples made with the same water.

	TCAA			DCAA		
	[Water] (μg/L)	[Tea] (μg/L)	Relative Increase (%)	[Water] (μg/L)	[Tea] (μg/L)	Relative Increase (%)
Experiment 1 [Cl] = 2.18mg/L	2.8 ± 0.9	5.2 ± 0.3	86	3.0 ± 0.8	18 ± 0.6	500
Experiment 2 [Cl] = 2.27mg/L	3.5 ± 0.1	6.1 ± 0.5	74	3.0 ± 0.5	24 ± 2.9	700
Experiment 3 [Cl] = 2.24mg/L	3.9 ± 0.4	7.2 ± 1.1	85	2.7 ± 0.3	20 ± 2.4	660
Averages	**3.4 ± 0.50**	**6.8 ± 0.60**	**82 ± 6.7**	**2.9 ± 0.50**	**21 ± 2.0**	**620 ± 110**

Table 2.3 The average TCAA and DCAA concentrations in triplicate analyses of tap water and hot tea. The residual chlorine concentration in the tap water used and percent increases of TCAA and DCAA are also indicated.

Table 2.3 indicates the similarity in average relative increases of TCAA and DCAA between brewing of coffee and hot tea. It is important to note that the standard deviation of observed concentrations is much lower in the tea analyses. Therefore the standard deviation of relative increase of TCAA and DCAA is also much lower. Increased confidence in this data lies in repeating the experiment several more times in order to see if we observe the same relative increases. Since sample analysis did not deviate among different beverages, it is unclear why there is greater variation in the analysis of coffee. It is possible that experimental error resulted in the higher variation of analysis. A plausible explanation for experimental error would be specific to the coffee making procedure. Variations in quantity of coffee grounds, exact water temperature, and water : coffee ground contact characteristics in the filter basket. These variables are especially difficult to control, and may have played a role in the variation seen in coffee analysis. The full data is available in Appendix A, Table 2.

The third and final beverage prepared with cold tap water was iced tea mix. The importance of analyzing this beverage rests in the fact that preparation of iced tea is markedly different from that of the two previous beverages. In the case of iced tea, the water introduced to prepare the beverage is cold, and the preparation itself primarily involves dissolution of a sugar-based powder into the water, rather than extraction of components into the water from a solid substance (as is done in coffee and tea preparation). Table 2.4 indicates the summarized results of iced tea analysis.

	TCAA			DCAA		
	[Water] (μg/L)	[Iced tea] (μg/L)	Relative Increase (%)	[Water] (μg/L)	[Iced tea] (μg/L)	Relative Increase (%)
Experiment 1 [Cl] = 2.27mg/L	3.5 ± 0.1	6.6 ± 3.5	89	3.0 ± 0.5	13 ± 7.4	320
Experiment 2 [Cl] = 2.21mg/L	2.7 ± 2.0	5.1 ± 0.2	89	3.6 ± 2.0	12 ± 2.2	230
Experiment 3 [Cl] = 2.14mg/L	2.1	6.6 ± 1.8	210	1.5	8.3 ± 3.0	450
Averages	**2.8 ± 1.1**	**6.1 ± 1.8**	**130 ± 69**	**2.7 ± 1.8**	**11 ± 4.2**	**330 ± 110**

Table 2.4 The average TCAA and DCAA concentrations in triplicate samples of tap water and iced tea. Residual chlorine concentration in the tap water and percent increases of TCAA and DCAA are also included.

Significant increases were detected in TCAA and DCAA concentrations after preparation of the beverage. In the case of iced tea, the increase in TCAA concentration is similar to that of coffee, but the average relative increase of DCAA is approximately half that of the previous beverages. There are a number of possibilities for the disparity in DCAA concentrations. It is possible that the difference in preparation methods affects the production of HAAs. The vastly different beverage composition means that iced tea mix is introducing different compounds to react with the residual chlorine compared to tea or coffee. Iced tea mix is primarily comprised of various sugar-based compounds, while coffee and tea are not. Possibly the sugar molecules react differently with the residual chlorine compared to other organic molecules. As mentioned in Chapter 1 (Section 1.4.2), the total organic carbon concentration is a good indicator of the amount of THMs and other DBP precursors present (Singer and Chang, 1989). In addition to this, Krasner (1999) notes that differences in the composition of the natural organic matter (NOM) will also affect the formation of DBP precursors in water. For example, waters with higher fractions of humic substances (generally large aromatic structures) were found to have higher DBP formation potentials (DBPFPs) (Reckhow, Singer, and Malcolm, 1990; Owen, Amy, and Chowdhury, 1993; Krasner et al., 1996). This finding in natural waters suggests that if the beverage contributes compounds more likely to react with chlorine to form DBPs, the relative increase of DBPs will be higher for that beverage.

It is also possible that the different reaction conditions occurring between the different beverages may play a role in the type and amount of compounds formed. For example, some researchers suggest that higher temperatures cause degradation of TCAA (Weisel et al., 1999; Froese et al., 2002). In addition, Summers et al. (1996) noted that temperature caused a variable effect on specific DBP formation (especially for THMs and HAAs). With this in mind, it is possible that the high water temperatures involved in brewing coffee or tea are causing degradation of some of the TCAA already present in the source water, or the newly formed TCAA. While this hypothesis was not thoroughly tested, it is interesting to note that the average change seen in iced tea (130% ±69%) using cold tap water was greater than that of both coffee (110% ± 76%) and tea (82% ± 6.7%). A single factor Analysis of Variance (ANOVA) was used to look at the effect of beverage type on the observed percent increase in TCAA concentration. At the $\alpha = 0.005$ level of significance, there was no difference between the mean percent increase in TCAA concentration when different beverage substrates were used. All beverage types analyzed caused a similar mean increase in TCAA concentration.

A further issue that must be addressed when investigating the different reaction conditions present during the preparation of each beverage is the level of chlorine residual in the water when the organic compound contacts the water source. The difference in heating conditions used for each beverage may play a role in the level of remaining chlorine residual. Heating of water to brew coffee or boiling water to

make tea may cause a decrease in the level of residual chlorine. Water samples were run through the coffeemaker and then tested with our chlorine tester to determine the concentration of residual chlorine present. In addition, water was also boiled for five minutes and then tested for residual chlorine concentration to see if these values do indeed change. Table 2.5 shows the results obtained from this analysis.

Water Sample	[Residual Chlorine] (mg/L)
Tap water n=3	1.47 ± 0.07
Tap water from coffeemaker n=3	1.25 ± 0.04
Boiled water (5 minutes) n=3	0.59 ± 0.03

Table 2.5: Average residual chlorine concentrations (from triplicate analyses) of the different water sources used to prepare beverages. These values indicate total chlorine concentration in water. The full data set is available in Appendix A, Table A13.

As evidenced from the experimental results, there is a marked discrepancy in the residual chlorine concentrations between cold tap water and the boiled tap water. Clearly a good deal of the chlorine was eliminated during the boiling process. This was not as evident in the water run through the coffeemaker, since the water is heated but not necessarily boiled, and any boiling would be for a very short time frame. This finding helps to support the findings of changes in TCAA concentration among the different beverages (i.e. that there is a relationship between change in TCAA concentration and chlorine residual). TCAA concentration in coffee increased 110% ± 76%, 130% ± 69% in iced tea, and only 82% ± 6.7% in tea. Reckhow and Singer (1985) found that a higher chlorine dose favors the formation of tri-halogenated compounds. With this in mind, it is possible that the different residual chlorine concentrations present are affecting the level of TCAA increase in the different beverages.

2.4.1.2 Analysis of Beverages Prepared With Milli-Q Water

Coffee, tea, and iced tea were all prepared using Milli-Q water in order to determine if the reduced levels of chemical compounds in the purified water source would have an impact on the production of TCAA and DCAA. It was presumed that the reaction of residual chlorine with organics in the beverages was contributing to the increase in TCAA and DCAA. Since Milli-Q water has a minimal level of residual chlorine, we expected to see minimal increases in TCAA and DCAA relative to the tap water samples. Using our residual chlorine tester, the Milli-Q water showed residual chlorine concentrations of approximately 0.03 mg/L (unreliable since this value is below our method detection limit) to 0.1 mg/L. Since chlorine was still available to react, increases in TCAA and DCAA concentrations were expected (but not to the same extent as those observed in the tap water samples). Table 2.6 shows the averages of the analyses of beverages prepared with Milli-Q water. Table 4 in Appendix A shows the full data set obtained from this analysis.

Beverage	TCAA			DCAA		
	[Milli-Q] (μg/L)	[Beverage] (μg/L)	Relative Increase (%)	[Milli-Q] (μg/L)	[Beverage] (μg/L)	Relative Increase (%)
Coffee	0.12 ± 0.02	0.23 ± 0.05	88	0.38 ± 0.11	3.27 ± 0.09	752
Tea	0.09	0.14 ± 0.09	55	0.38 ± 0.11	1.79	371
Iced Tea	0.10 ± 0.02	0.12 ±0.01*	20	0.38 ± 0.10	1.11 ± 0.33	192

Table 2.6 The average TCAA and DCAA concentrations found in triplicate samples of Milli-Q water and beverages made with the same Milli-Q water. Percent increases after beverage preparation are also indicated.

- **Only duplicate measurements were available for this analysis, therefore standard deviation was estimated using the calculation $s = (\Sigma d^2 / 2k)^{1/2}$, where d is the difference between duplicate measurements, and k is the number of duplicate measurements.**

While the concentrations of TCAA and DCAA are clearly lower in the analyzed beverages, the relative increase for each HAA is still similar to those reported for tap water. The increases for coffee were very similar, while those reported for iced tea were somewhat lower. This data suggests a number of possibilities with respect to the hypotheses. The first possibility is that chlorine is not the only limiting factor for the production of TCAA and DCAA during beverage preparation. If it were, then we would expect to see lower relative increases during beverage preparation due to the much lower levels of residual chlorine in the Milli-Q water. Therefore, it is possible that there is some chemical constituent inherent to the beverages themselves that contribute to the level of TCAA and DCAA in the samples. Krasner et al. (1996) suggests that the composition of natural organic matter will dictate the DBP formation potential. It is possible that the compounds introduced by the beverages have very high formation potentials, therefore they are readily reacting with any available chlorine to form TCAA and DCAA.

It should be noted that the original TCAA and DCAA concentrations are very low, therefore it takes little additional production of these DBPs to show a marked relative increase. Theoretically, doubling or tripling a small amount of a given chemical product would require a very minimal amount of precursors, in this case residual chlorine and organic compounds. Minor differences in the reaction conditions, such as residual chlorine concentration, could markedly affect the relative increase due to the very low starting concentrations. Finally, it is also possible that there is a low

level of TCAA and DCAA present in the coffee, tea, and iced tea and the Milli-Q water is simply extracting them. A very small amount of these DBPs in the beverages themselves could substantially affect the relative increase. This theory is tested in Section *2.4.1.4.*

2.4.1.3. Analysis of beverages prepared with water from a filtration pitcher

Tap water was treated by filtration through a "PUR Plus" water filtration pitcher, which employs an activated carbon filter to remove chlorine and other compounds (such as DBPs) from the tap water. These filters are commercially available and are effective in reducing the chlorine residual found in tap water. It was expected that the filters would reduce residual chlorine levels and, possibly, TCAA and DCAA found in the tap water. Analysis of this water source was carried out to determine if the lower chlorine residual and reduced number of chemical components in the water would affect the formation of TCAA and DCAA when beverages were prepared. Analysis of the water purified through the filtration pitcher had a residual chlorine concentration in the range of 0.46mg/L to 0.59mg/L. Therefore, filtration removed approximately 75% of the residual chlorine found in Edmonton tap water (which is usually in the range of 2.0mg/L to 2.3mg/L). Table 2.7 gives an indication of the TCAA and DCAA concentrations found in the filtered water and coffee brewed using this water. Table A5 in Appendix A provides all of the data obtained for this analysis.

Beverage	TCAA			DCAA		
	[Water] (μg/L)	[Beverage] (μg/L)	% Increase	[Water] (μg/L)	[Beverage] (μg/L)	% Increase
Coffee	2.6 ± 1.2	3.4 ± 0.9	31	3.3 ± 1.3	10 ± 2.5	210
Coffee	2.8	4.0 ± 1.2	43	2.3	12 ± 4.0	430
Averages	**2.7 ± 1.2**	**3.7 ± 1.1**	**37 ± 8.5**	**2.8 ± 1.3**	**11 ± 3.3**	**320 ± 160**

Table 2.7. The average TCAA and DCAA concentrations in triplicate samples of water processed through a PUR Plus activated carbon water filtration pitcher, and concentrations of TCAA and DCAA in coffee prepared with this water. Relative increase (percent) of TCAA and DCAA after coffee preparation are also indicated.

As shown in the above table (Table 2.7), the tap water filtered using the filtration pitcher did not show marked reductions in the levels of TCAA and DCAA. It appears clear that the filtration process did not remove much of the TCAA and DCAA inherent to the tap water (that was essentially formed during the water treatment process). It is interesting, however, to note the reduced production of TCAA and DCAA during coffee preparation (compared to the results found for tap water and Milli-Q water). The filtered water data supports the hypothesis that residual chlorine plays a role in the formation of TCAA and DCAA. It appears that filtration does not remove the TCAA and DCAA already present in tap water, but it does help to reduce formation during beverage preparation. The most plausible explanation for this is the reduction of residual chlorine available for reaction with the added organics.

The observed data from the water filtered through a filtration pitcher suggests that components inherent to the beverages may be contributing to the overall concentration of TCAA and DCAA found in the beverage samples. The relatively low chlorine residual found in the filtered water implies that minimal TCAA and DCAA formation (via oxidative reactions) will occur. As is reported for the case of Milli-Q water, increases are noted in the concentrations of these HAAs. The increases are not as profound as those reported in Milli-Q water, but they are still present. The smaller increases observed in the coffee prepared with filtered water is the key factor supporting the suggestion that the beverages are contributing to the level of TCAA and DCAA in the water. It seems unlikely that significant formation

of TCAA and DCAA would be observed when water with low chlorine residual is used to prepare beverages, yet increases are still observed in our experiments. If we attribute increased DBP concentration solely to the reaction of chlorine with organic compounds in beverages, water with low chlorine residual (i.e. Milli-Q water or water from a filtration pitcher) would not present large increases due to lack of reactants. Contribution of DBPs or DBP precursors from components in the beverage mix could explain this phenomenon. In this case, we would expect to see a relatively standard increase of TCAA and DCAA. Beverage preparation and analysis was standardized, therefore increases contributed by the beverage would not vary greatly. Based on the very low levels of TCAA and DCAA in the Milli-Q water, this addition of TCAA and DCAA would appear as a large relative increase. When comparing to a very low original level of DBPs, a minimal increase contributed by the beverage themselves would present as a substantial relative increase. The smaller increases seen in the filtered water samples may be attributed to the fact that the base level of TCAA and DCAA is higher than that of Milli-Q, therefore the relative increase due to beverage contribution does not appear as profound. The original levels of TCAA and DCAA in the water from the filtration pitcher are much higher than those reported in Milli-Q, thus the relative relative increase based on a fixed increase would appear smaller for the water from a filtration pitcher.

2.4.1.4. Investigating the presence of TCAA and DCAA in the beverage substrates

Preparing beverages with HPLC grade water that contains no residual chlorine will illustrate whether the beverage substrates are contributing to the TCAA and DCAA concentrations. Since there is no available chlorine in the water used, any detected TCAA and DCAA is coming from the beverage matrix. Analysis of HPLC grade water and coffee prepared with this water showed that all samples had TCAA and DCAA concentrations below the MDL. The experimental results did not provide evidence supporting the hypothesis that the beverage substrates are contributing TCAA and DCAA. Confidence can't be placed on the experimental values, since they are lower than the MDL. However, concentrations that low suggest that the beverage substrates are not sufficiently contributing HAAs to affect our relative increases. Levels that close to the MDL suggest minimal levels of HAAs, levels that would be too small to affect the relative increases.

2.4.2. The association of residual chlorine with TCAA and DCAA formation

Water samples containing a range of free chlorine concentrations were used to prepare coffee and iced tea in order to determine if there is an association between the two. Singer (1994) notes that the rate, extent, and distribution of DBP formation is affected by chlorine dose, and higher doses favor the formation of HAAs. With the extensive amount of organic compounds available to react, increasing dose should cause an increase in the concentration of TCAA and DCAA. In one experiment, water sources containing a range of chlorine residual (spanning from 0.05 mg/L to 13 mg/L) were used to brew coffee. Figure 2.2 shows the linear relationship between residual chlorine and TCAA formation in coffee, while Figure 2.3 shows the relationship observed for DCAA formation in coffee. The results show a positive linear relationship between residual chlorine and the formation of TCAA and DCAA in the coffee. Graphical analysis of the relation between residual chlorine and TCAA and DCAA concentrations showed $R^2_{TCAA} = 0.9989$ and $R^2_{DCAA} = 0.9787$. Based on the strong linear associations found, there is strong evidence that supports the hypothesis that residual chlorine plays a major role in the formation of TCAA and DCAA in beverages. All of the TCAA and DCAA concentrations for coffee brewed with Milli-Q water containing different residual chlorine concentrations are provided in Appendix A, Table A8.

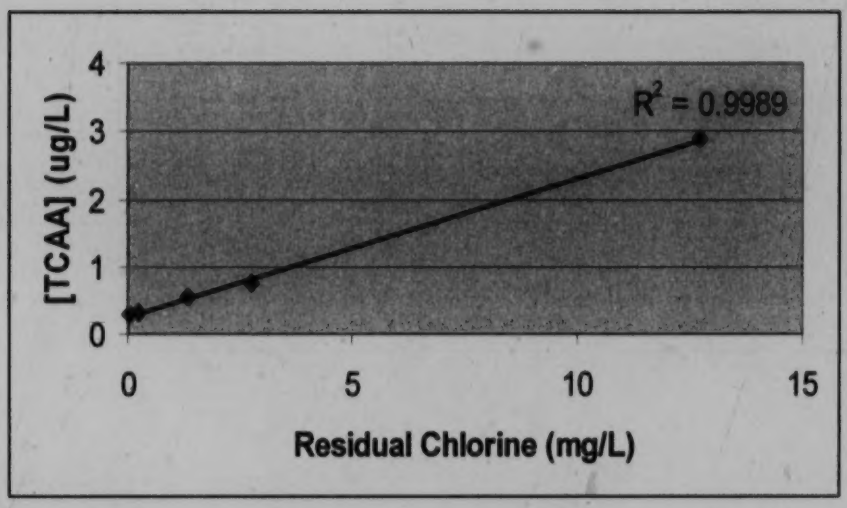

Figure 2.2 The relationship between residual chlorine concentration and TCAA concentration in coffee. Samples at each level were analyzed once.

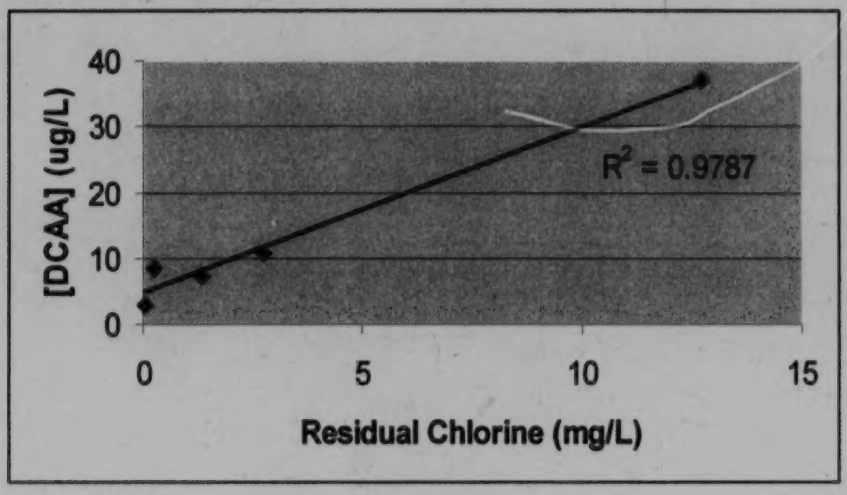

Figure 2.3 The relationship between residual chlorine and DCAA concentration in coffee. Samples at each level were analyzed once.

The graphs clearly illustrate the relationship between residual chlorine levels and the production of TCAA and DCAA in coffee. It is important to note the detection of TCAA and DCAA at the lowest concentrations of chlorine residual. Based on the data obtained from preparing coffee using HPLC grade water, the lack of a zero intercept is presumably the result of reaction between the available chlorine in the Milli-Q water and the organics in the beverages. Experimental data suggests that the beverage substrate is not contributing to the overall TCAA concentrations.

Similar results are noted for the preparation of iced tea using water containing a range of chlorine residual concentrations. In this experiment, the range of chlorine concentration spanned from 0.09 mg/L to 4.20 mg/L. Again the results indicate a positive linear relationship between TCAA and DCAA concentrations in iced tea and residual chlorine concentration in water. From this experiment, $R^2_{TCAA} = 0.94$ and $R^2_{DCAA} = 0.95$. Figure 2.4 indicates the relationship between residual chlorine concentration and TCAA concentration in iced tea, while Figure 2.5 indicates the relationship between residual chlorine concentration and DCAA concentration in iced tea. As provided for the coffee analysis, Appendix A (Table A6b) shows all of the values obtained for this analysis.

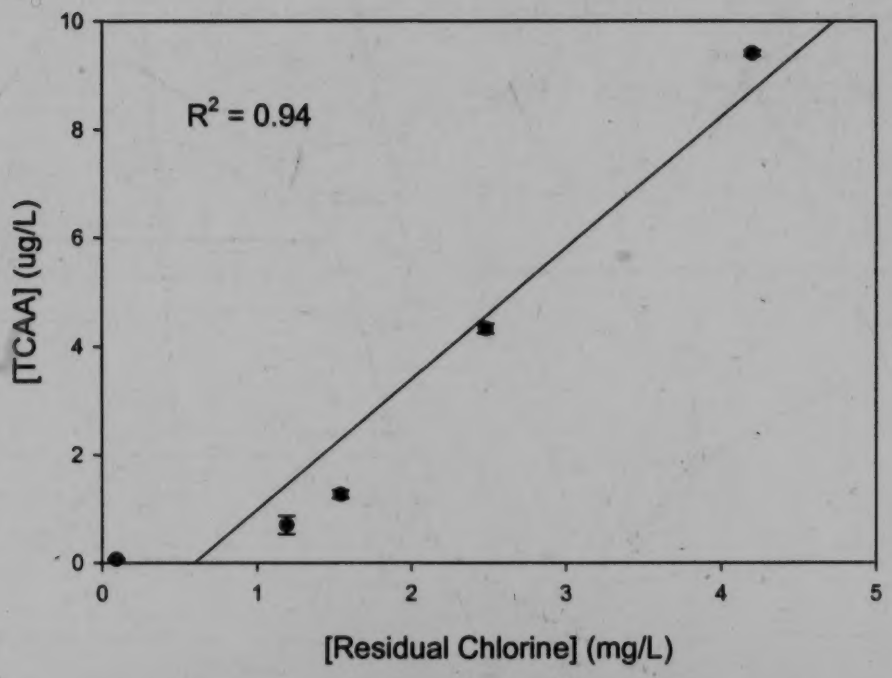

Figure 2.4 The relationship between residual chlorine concentration and TCAA concentrations in iced tea. Each point is an average of duplicate analyses.

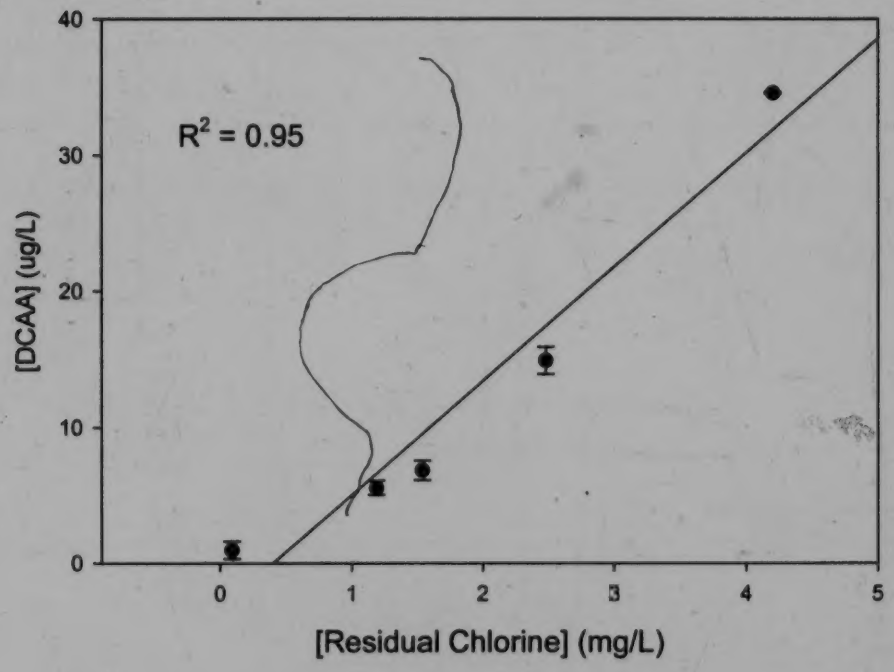

Figure 2.5 The relationship between residual chlorine concentration and DCAA concentrations in iced tea. Each point is an average of duplicate analyses.

Analysis of both coffee and iced tea samples indicate strong linear relationships between residual chlorine concentration and the formation of TCAA and DCAA. These data suggests that residual chlorine plays a large role in the formation of TCAA and DCAA when beverages are prepared.

2.5 Conclusions

The SPME method coupled with GC-ECD provided highly reproducible results for the detection of TCAA and DCAA in water and beverage samples. Analysis of the acquired data suggests that beverage preparation contributes to the concentrations of both TCAA and DCAA, as the beverage samples consistently showed higher concentrations of these chemicals compared to the water used to make them. In addition, the positive linear relationship between residual chlorine concentration in water and the concentration of TCAA and DCAA in coffee and iced tea supports the hypothesis that organics in the beverages are reacting with excess chlorine to create additional HAAs. The increases observed when using Milli-Q water or water run through a water filtration pitcher may still very well be due to the residual chlorine left in these water samples. As shown from analysis of the residual chlorine concentrations, there was still chlorine left over in these water samples. The TCAA and DCAA concentrations were present in these waters at very low levels (μg/L concentrations). Presumably, causing a 2-3X increase in these concentrations would not require much chlorine residual. There is probably sufficient chlorine available in Milli-Q water and the water filtered through the filtration pitcher to react with the introduced organics to create additional TCAA and DCAA (such that the concentrations could double or triple).

Alternatively, the fact that we still see increases in the beverage samples prepared with Milli-Q and filtered water suggests that the TCAA and DCAA concentrations might be increased by something inherent to the beverages. It is possible that the beverages contain TCAA and DCAA, and are easily extracted once introduced to an aqueous environment. Experiments conducted to test this theory suggest that the beverages do not contribute to the TCAA and DCAA concentrations. Therefore, it appears that the overall TCAA and DCAA concentrations in beverages are ultimately affected by the following factors:

$$(TCAA+DCAA)_{Total} = (TCAA+DCAA)_{Existing} - (TCAA+DCAA)_{loss} + (TCAA+DCAA)_{Formation}$$

Originally, it was suggested that the beverage substrate might be contributing to overall HAA concentrations, but this claim could not be supported experimentally. Matrix effects causing increased TCAA and DCAA detection due to nonspecific volatile organics binding to the SPME fiber seem unlikely as well. We did not observe substantial increases in the baselines during chromatographic analysis, and it is unlikely that matrix effects would only occur at the TCAA and DCAA peaks. If anything, competitive binding with nonspecific compounds would provide TCAA and DCAA concentrations lower than the actual value. Therefore, the TCAA and DCAA increases seen in beverages prepared with Milli-Q water and tap water processed

through an activated carbon filter is mainly due to the residual chlorine still available after the filtration process.

This research has provided information suggesting that human exposure to TCAA and DCAA may be significantly higher than what is presumed using tap water ingestion as a surrogate measure of exposure to TCAA and DCAA. There is reason for further investigation in order to obtain more accurate estimates of human exposure to TCAA and DCAA. At present, it is reasonable to suggest that human exposure estimates that do not differentiate sources of water ingestion may be inaccurate. The increases observed during beverage production serve to support this statement. In addition, since approximately 2/3 of all water is ingested via beverages (EPA, 1997), it is reasonable to assume that TCAA and DCAA exposure is being underestimated. This dilemma will only be rectified by further examination of TCAA and DCAA concentration in foods and beverages.

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Chapter 3: Application of results to Adelaide trial

3.1 Introduction

The ability to establish a credible link between DBP exposure via drinking water and adverse human health effects lies in the implementation of valid epidemiological studies. To date, a number of epidemiologic studies have been carried out to investigate this problem. The majority of studies performed have looked at DBP exposure and cancers, namely colon cancer (Cragle et al., 1985; Marrett et al., 1995; Hildesheim et al., 1998), rectal cancer (Gottlieb et al., 1982; Hildesheim et al., 1998), and bladder cancer (Cantor et al., 1987; McGeehin et al., 1993; King et al., 1996; Freedman et al., 1997; Cantor et al., 1998). More recently, a number of studies have addressed the association of DBP exposure with adverse reproductive outcomes (Kramer et al., 1992; Savitz et al., 1995; Swan et al., 1998; Waller et al., 1998; Dodds et al., 1999; Klotz and Pyrch, 1999; Magnus et al., 1999; King et al., 2000; Nieuwenhuijsen et al., 2000; Yang et al., 2000). While there have been a number of epidemiolgic studies performed, a clear association has not been established between DBP exposure via drinking water and adverse human health effects: The primary limitation of the studies performed is the inadequate assessment of individual exposure (Swan and Waller, 1998; Weisel et al., 1999; Backer et al., 2000; Nieuwenhuijsen et al., 2000).

Epidemiologic studies addressing this issue need to be strengthened by obtaining a more accurate measure of exposure to individual DBPs suspected of causing adverse health effects. Validating a biomarker of exposure to DBPs has received increasing attention as a viable means of improving human exposure assessment. Froese et al. (2002) have suggested that a valid biomarker of exposure has a great deal of potential for improving exposure assessment for DBPs. Biological samples containing a compound that can be readily measured and that reflect the presence of other DBPs enhances our confidence in what compounds a human is truly exposed to through our water supply. The compounds that are detected are measured in an individual body fluid rather than assuming they enter the body based on extrapolations from analysis of water samples.

Urinary TCAA has been assessed as a biomarker for exposure in three exposure studies (Weisel et al., 1999;; Bader, 2001 (MSc. Thesis); Froese et al., 2002). The first study (Weisel et al., 1999) reported a significant association between TCAA ingestion exposure and TCAA urinary excretion ($R^2 = 0.575$, $P < 0.0001$; creatinine-normalized concentration: $R^2 = 0.603$, $P < 0.0001$ for a cross-sectional analysis), suggesting the validity of using urinary TCAA as a biomarker of exposure to DBPs. Weisel et al. (1999) also reported, however, that there was very poor correlation between TCAA excretion and the concentration of TCAA in tap water ($R^2 = -0.04$, $P = 1$). Clearly using water concentration as a surrogate for exposure was not possible in this study. Weisel et al. (1999) mentioned that this poor correlation may be due to

the large variability in the TCAA concentration from different water sources, the amount of chlorinated water ingested, and consumption of beverages prepared from water. In addition, the study design implemented (a 48-hour recall questionnaire concerning previous water consumption administered to participants) may have added to uncertainty in the results. Retrospective studies can lead to recall bias whereby the participant over- or underestimates the amount of water ingested. Hennekens and Buring (1987) mention that individuals who are conscious of the fact that they are involved in a study are more likely to remember experiences differently. Participants in the Kim and Weisel study knew of the study, and had already participated in an epidemiologic study performed by Klotz et al. (1996). Therefore these individuals may either exaggerate their ingestion if they believe it to contribute to any potential adverse effect that they suffered, or they may minimize their exposure estimates from a belief that this may be more acceptable to the investigators.

Weisel et al. (1999) found a much higher correlation between TCAA exposure and excretion when they accounted for the amount of water consumed, the proportion of heated water consumed (where they estimated a 39% reduction in TCAA due to boiling), and the use of home water filters (estimating a 70% reduction for any filter type). These results suggested that accurate exposure classification is dependent on obtaining a comprehensive measure of exposure from all potential sources. For instance, in a study in which ingestion is the dominant means of exposure, accurate exposure classification involves taking into account all means of ingestion, including

water, beverages, and food (Weisel et al., 1999). This premise supports the merits of analyzing the effect of increased DBPs in beverages on exposure measures.

Froese et al. (2002) performed a pilot study in Adelaide, Australia to identify the feasibility of using TCAA as a biomarker of exposure. In order to minimize potential recall bias from a retrospective study, they implemented a prospective study using daily exposure diaries for 10 participants. The investigators were able to collect detailed ingestion diaries from 10 volunteers over the course of the study in order to establish a more accurate measure of tap water consumption in the study group. The longitudinal study design was implemented to get a better estimate of personal exposure by looking at individuals over time. The cross-sectional study performed by Kim and Weisel (1999) looked at individuals at one point in time, and could not obtain as reliable an estimate of personal exposure to TCAA. Factoring in the different sources of water based on where water was ingested, whether water ingested was cold or hot (i.e. hot water refers to beverages such as tea or coffee where a decrease in TCAA concentration of 35% was assumed), and ingestion of other beverages (referring to commercially prepared beverages which were not factored in since no exposure amount could be estimated), Froese et al. (2002) obtained good correlation between measured TCAA ingestion and excretion ($R^2 = 0.90$, $P < 0.005$, n = 9 for one study day).

Froese et al. (2002) also reported a high level of inter- and intra-individual variability in both TCAA ingestion and excretion. Three participants showed TCAA ingestion ranging from 41 to 73 μg/d, while the other seven had more than a ten fold lower level of ingestion, ranging from 2.3 to 7.9 μg/d. Variability in this measure was attributed to the TCAA concentration in the different source water (tap water TCAA concentrations ranged from 1.1 μg/L to 49 μg/L for the different participants homes) and volume of water consumed. In addition, the proportion of water ingested from different sources (i.e. home, work, and hot or cold water) showed significant variability. Individuals showed RSDs of 30 to 300% for any particular category of water consumption over 12 days of study. Overall, total cold tap water consumption at home accounted for 39 ± 17% of all ingestion, and cold tap water consumption at work accounted for 7 ± 9% of total consumption. Hot beverages consumed at home accounted for 10 ± 6% of all ingestion, and "other" beverages accounted for approximately one-third of all water ingestion. To date, studies have concentrated on DBP concentrations in cold tap water. Since almost 50% of TCAA is ingested through sources other than cold tap water, there is clearly a deficiency of information with regard to TCAA ingestion.

Froese et al. (2002) indicated that TCAA excretion showed high variability as well, ranging from 2.4 to 5.8 μg/d (individual average excretion) for nine of the participants, and one other participant showing an average of 24 μg/d. Overall, the original data obtained from the Adelaide study estimated excretion ranging from 15%

to 71% of ingested TCAA. The high variability in percent TCAA excreted possibly reflects inter-individual variability in metabolism of TCAA, while the numeric variability could also be affected by amount of TCAA ingested. These findings reflect the difficulty in obtaining an accurate measure of TCAA exposure through the use of urinary TCAA as a biomarker. A clearer picture of TCAA ingestion will help clear up the unknowns with regard to variability in excretion.

The considerable inter- and intra-individual variability in TCAA ingestion and excretion discovered by Froese et al. (2002) indicates the need to clarify some of the unknowns with regard to exposure classification. Routine water quality monitoring data was found inadequate in assessing the amount of DBPs being introduced to an individual. A direct approach that measures an internal dose is necessary to improve exposure classification, but steps can be taken to further improve the approach applied by Weisel et al. (1999) and Froese et al. (2002). Based on the diaries obtained from Froese et al. (2002), a good deal of water is ingested via beverages and hot water (referring to tea or coffee). Chapter two has already indicated that the concentration changes significantly when beverages are prepared, therefore this data, when applied to an exposure diary, should affect overall DBP exposure. Assuming a 35% reduction in TCAA when hot beverages are prepared is inaccurate. I have shown an increase in TCAA levels during the production of beverages. Therefore it will be useful to apply this new data to investigate the impact of this information on the overall TCAA ingestion profiles. I want to discover whether or not the beverage

data, when applied, will affect the overall exposure estimates, and intra- and inter-individual variability in TCAA exposure. The effect of beverage DBP concentrations will shed light on the validity of using TCAA as a biomarker of exposure. I want to increase our confidence in TCAA exposure estimates by showing the effect of beverage data on the overall exposure to TCAA.

Using the experimental data that illustrated increases in TCAA concentration during coffee preparation (Chapter 2), average relative increases found in brewed coffee were applied to the available exposure diaries and TCAA exposure was recalculated. Originally, TCAA exposure in boiled water (presumably corresponding to coffee and tea ingestion) was multiplied by a factor of 0.65 to account for the assumed 35% reduction of TCAA in boiled water. It was expected that exposure might change dramatically for those individuals who ingested large volumes of boiled beverages when we used our information that suggested significant increases in TCAA during beverage production. Multiplying by a factor of 2.1 (the overall average TCAA concentration increase in coffee) rather than 0.65 will substantially change TCAA exposure in an individual who ingests large volumes of boiled beverages.

Based on the exposure diary data provided in Appendix B, hot water ingestion (referring to tea or coffee) compared to total water ingestion ranged from 0% to 51% for all of the participants. In addition, 6 of the 10 participants consumed approximately 20% of their fluids as hot water. Clearly there is a substantial amount

of water from boiled beverages, and changes to TCAA exposure through boiled beverage ingestion should be explored. Furthermore, this study was performed in Australia during the summer, which correlates with hot temperatures (30-40^{0}C). It seems reasonable to presume that hot beverage ingestion would increase during colder periods of the year. Overall, it appears important to test the effects modifying the estimated TCAA by applying the data obtained in Chapter 2.

3.2 Objective

A major challenge in assessing DBP exposure is the fact that exposure is multiroute. A large amount of uncertainty in exposure assessment arises from the different water sources (that all contain varying levels of DBPs) that individuals ingest. Because of the large number of potential DBP sources, it would be an asset to exposure assessment to obtain a biomarker of exposure that will provide an accurate indicator of DBP exposure in an individual, regardless of exposure route. Obtaining a valid biomarker requires extensive knowledge of the exposure to that compound, and how the chosen marker reflects exposure to other DBPs. The work of Kim et al. (1999), Weisel et al. (1999), and Froese et al. (2002) have all shown TCAA as a promising biomarker of exposure to DBPs. Detailed exposure information has been collected by Froese et al. (2001), which ultimately increases our confidence in exposure assessment for TCAA. Applying the beverage data obtained experimentally (Chapter 2) to the exposure diaries collected by Froese et al. (2002) will help in clarifying

TCAA exposure assessment. The aim is to identify if the increased TCAA exposure affects inter- and intra-individual variability, and I want to see how much overall exposure can be affected by making adjustments to exposure based on my experimental findings. Seeing the effects of the beverage data will help to show the accuracy of present TCAA exposure estimates and will also suggest whether we need to scrutinize the ingestion of hot and cold beverages when trying to obtain an accurate measure of exposure in a study population.

3.3 Materials and Methods

- **All materials and methods data for the original study are taken from Froese et al. (2001).**

3.3.1 Recruitment of Volunteers

Volunteers for the pilot study were all healthy adult volunteers who worked at the Australian Water Quality Centre (AWQC). In the Adelaide, South Australia metropolitan region, there are 6 major supply zones for 6 treatment plants. Since each treatment plant took water from a separate reservoir, the differences in water shed characteristics and dissolved organic matter will affect the nature of the DBPs from each water supply. As a result, the study aimed to obtain at least 2-3 volunteers who received water from 4 different treatment plants (in order to obtain a representative sample of the different water supplies). Froese et al. (2002) note that "the study protocol was approved by the Monash University Standing Committee on

Ethics in Research Involving Humans and written informed consent was obtained from each of the participants at enrolment".

3.3.2 Study Design

Participants were required to maintain a diary in which ingestion of all water was logged, divided into the categories cold, hot, and other. Hot water referred to all beverages that were prepared using boiling water, such as coffee and tea. Other beverages included any commercially prepared beverages, such as bottled water, soft drinks, and alcoholic beverages. In addition, exposure to DBPs via all other major water contact was documented (such as swimming, bathing or showering, and using water to wash items). Finally, potential exposure to trichloroethylene or trichloroethane, known precursors to TCAA (Breimer et al., 1974; Fisher et al., 1991; Humbert et al., 1994), were tracked by noting visits to drycleaning establishments. These diaries were begun 48 hours prior to the first urine sample collection.

The samples analyzed included entire first morning urine (FMU) voids as well as daily home tap water samples. Samples were analyzed within 6 hours of collection, and any samples that had to sit for four hours or more were stored in coolers with ice packs.

3.3.3 Analysis of Samples

Analysis of TCAA was carried out using a modified version of USEPA Method 552.2 (USEPA, 1995), and the method used by Kim and Weisel (1998). 40mL urine samples were measured into 50mL polycarbonate centrifuge tubes and 80μL of a 25μg/mL solution of 2,2-dichloropropionic acid was added as a surrogate standard. Samples were acidified using 2mL of concentrated H_2SO_4. Approximatley 12g of sodium sulfate and 4mL of methyl-*tert*-butyl ether (MTBE) was added. Finally, approximately 500μg/L of 1,2,3-trichloropropane was added (as an internal standard). Samples were hand shaken for 8 minutes to ensure full salt saturation, and subsequently centrifuged at 2500 rpm for 15 minutes.

Following this, the entire organic layer was extracted using Pasteur pipettes and placed in 10mL glass vials. The nonvolatile HAAs were derivitized by adding 3mL of acidified methanol (10% H_2SO_4 in methanol), vortexing for 30s, and placed on a heating block set at 50^0C for 1 hour. After this step, the acid was neutralized with 8mL of saturated $NaHCO_3$. The solvent layer was extracted and eluted through a disposable activated carbon SPE column (6mL x 250mg: Envi-Carb, Supelco) to remove organic contaminants. The eluate was collected in 2mL autosampler vials for analysis.

Tap water samples were prepared in the same manner, with the exception that the Envi-Carb SPE step was omitted.

Samples were analyzed using a Varian 3400 GC with a single injection (run on splitless mode) leading into two analytical columns (DB-1, 30m x 0.25mm i.d.; 25μm film / DB-1701, 30m x 25mm i.d.; 25μm film) with an electron capture detector (ECD). The two different columns had very different polarities, leading to different elution order of the compounds. This enabled confirmation of the peaks eluted. Calibration was carried out using a mixture of methyl ester HAAs, and the TCAA calibration range was ≤ 0.1 to 50μg/L.

The relative standard deviation on 11 triplicate analyses of tap water gave a relative standard deviation of 4.8%, and triplicate analyses of 17 urine samples gave a relative standard deviation of 8.5%. The method detection limits were found to be 0.2μg/L for tap water and 0.3μg/L for urine, based on three times the standard deviation for triplicate analyses, averaged for triplicate sets with TCAA ≤ 2μg/L.

Since chloral hydrate is a precursor for TCAA, tap water samples were also analyzed for this compound. Analysis was based on the AWQC Standard Test Method TMS-003 "Chlorination disinfection by-products (haloacetonitriles, chloroketones, chloropicrin, chloral hydrate) in water", which is based on USEPA Method 551 (USEPA, 1995).

3.3.4 Application of beverage data to the acquired Adelaide data

- *This methodology involves application of our beverage analyses to the data acquired by Froese et al. (2002) using the previously described protocol.*

Given the detailed exposure and urinary excretion data available from the Adelaide pilot study (Froese et al., 2002), we had the opportunity to apply our beverage data in order to determine how these new findings might influence the conclusions from that study. Since the most extensive analysis was performed on coffee, the values for relative increase of TCAA were obtained from the coffee analysis. Using the recorded relative increases for all analyses of coffee samples, the average value for relative increase in TCAA during coffee preparation was 110 ± 76%, so the average, high and low estimate (i.e. 110%, 186%, and 34%) were applied. The high standard deviation reported in relative increase is attributed to the fact that TCAA concentration in Edmonton tap water is very low (approximately 3 – 8 μg/L), therefore even a small variation in increased TCAA concentration during coffee preparation will have a substantial effect on the relative increase. All TCAA exposure from boiled beverages were multiplied by 1.34, 2.10, and 2.86 (based on low, average, and high exposure). These values are in marked contrast to the previously applied multiplier of 0.65 (which accounts for a 35% decrease in TCAA in boiled water).

Using beverage data, we could investigate the difference in TCAA exposure for the volunteers. In addition, we were able to apply these data to the excretion versus ingestion analysis to see how the data was affected. Finally, the TCAA half-life calculations were also modified to illustrate the increase in duration of TCAA existence in the body following exposure. All results in this section refer to the newly generated data.

3.4 Results and Discussion

3.4.1 Assessing the extent of TCAA ingestion

The mean ± 1 standard deviation values for TCAA increase when coffee is brewed were applied to the "boiled water" ingestion data from the Adelaide trial study. As might be expected, for those individuals who consumed large amounts of boiled water, the increase in calculated TCAA ingestion was quite substantial. Table 3.1 illustrates the increase in TCAA ingestion, both numerically and percentage wise, for all three exposure categories. For those individuals who ingested either a large amount of boiled beverages or a large proportion of boiled beverages (relative to total ingestion), the increases in calculated TCAA ingestion were profound. Estimated TCAA exposure can increase by up to 180%, as shown by the calculated TCAA ingestion increases for participant seven. It should be noted that boiled beverages comprised approximately 51% of this individual's fluid consumption. As mentioned earlier, a high proportion of fluid consumption from hot beverages will result in a

greater increase in overall TCAA consumption. The other notable increases are seen in participant four, showing increases in TCAA exposure from 25% to 75% and participant nine, showing approximate increases in TCAA exposure ranging from 33% to 110%. Participant four consumed a large volume of boiled beverages, while participant nine ingested a moderate volume, but high proportion of boiled beverages relative to total beverage consumption. Table 3.1 illustrates the large variation of increases in numeric amount and relative increase from one individual to another, which ultimately affects TCAA-*in*. Table 3.2 shows the relative increase in TCAA ingestion for boiled beverages alone. Table 3.3 summarizes this information to show the changes in TCAA-*in* for boiled water ingestion and total TCAA-*in*. In addition, Table 3.4 provides the volumes of each type of fluid consumed and the location of consumption, which clearly illustrates the variation in fluid consumption, and ultimately TCAA ingestion, across the study population. Figure 3.1 illustrates how each volunteer is affected by applying the beverage data. This graph shows the disparity in overall estimated TCAA exposure, and additionally illustrates how individuals are affected differently based on the proportion of boiled beverage consumption. It is clear that TCAA ingestion (and increases in TCAA ingestion) are varied among the study population. This emphasizes the difficulty in assessing DBP exposure from water quality data.

Participant	Original TCAA-*in* Estimate (μg)	TCAA increase (low exposure estimate)		TCAA increase (average exposure estimate)		TCAA increase (high esxposure estimate)	
		(μg)	%	(μg)	%	(μg)	%
1	510	2.5	0.49	5.0	0.98	7.6	1.5
2	890	0	0	0	0	0	0
3	110	10	9.7	22	20	33	31
4	630	150	25	330	52	500	80
5	49	11	22	22	45	34	69
6	71	0.35	0.49	0.74	1.0	1.1	1.6
7	32	18	56	37	120	57	180
8	44	5.3	12	12	26	18	41
9	230	77	33	160	69	250	110
10	59	9.2	16	20	33	30	50

Table 3.1: Numeric and relative increases for total TCAA ingestion for all participants in the Adelaide trial study. The three exposure categories are based on the low, average, and high relative increases observed when coffee is brewed (corresponding to relative increases of 34%, 110%, and 186%).

*** NOTE – Participant two reported no boiled beverage consumption during the study.**

Subject	TCAA Increase (Low exposure)		TCAA Increase (Avg. exposure)		TCAA Increase (High exposure)	
	(μg)	%	(μg)	%	(μg)	%
1	2.5	110	5.0	230	7.6	350
2	0	0	0	0	0	0
3	10	110	22	230	33	350
4	150	100	330	220	500	340
5	11	110	22	220	34	340
6	0.35	110	0.74	220	1.1	340
7	18	110	37	220	57	340
8	5.3	34	12	74	18	110
9	77	110	160	220	250	340
10	9.2	100	20	220	30	330

Table 3.2: Numeric and percent TCAA increases when looking at boiled water ingestion alone (assuming boiled water refers to coffee) for all participants in the Adelaide trial study. Each increase is calculated relative to the originally calculated values from the Adelaide study.

Subject	Boiled TCAA In (Orig.) (μg)	Total TCAA In (Orig.) (μg)	Boiled TCAA In (Low) (μg)	Total TCAA In (Low) (μg)	Boiled TCAA In (Avg.) (μg)	Total TCAA In (Avg.) (μg)	Boiled TCAA In (High) (μg)	Total TCAA In (High) (μg)
1	2.2	510	4.7	510	7.2	510	9.8	520
2	0	890	0	890	0	890	0	890
3	9.3	110	20	120	31	130	42	140
4	150	630	300	780	480	950	650	1100
5	9.8	49	20	59	31	70	43	82
6	0.33	71	0.68	72	1.1	72	1.5	73
7	17	32	34	49	54	69	73	88
8	16	44	21	49	27	55	33	61
9	72	230	150	310	230	390	320	480
10	8.9	59	18	69	29	79	39	89

Table 3.3: Summary of theoretical TCAA ingestion from boiled water consumption (assuming this is predominantly coffee) and total water consumption using the Adelaide water ingestion survey. Estimated values from the original survey are provided, as are low, average and high values derived from the data provided in Chapter 2. The low, average, and high exposure categories refer to an increase in TCAA concentration in beverages of 134%, 210%, and 284%, respectively.

Subject	Category Location	Home	Cold Work	Other	Home	Hot Work	Other	Other	Total
AD01									
	Avg	975	0	400	0	0	75	1700	3150
	s.d.	575	0	550	0	0	125	725	761
	RSD	59%		138%			167%	43%	24%
	n	10	10	10	10	10	10	10	10
AD02									
	Avg.	2150	0	0	0	0	0	1400	3550
	s.d	1050	0	0	0	0	0	300	900
	RSD	49%						21%	25%
	n	10	10	10	10	10	10	10	10
AD03									
	Avg	950	350	0	100	325	0	800	2500
	s.d	375	325	0	125	325	0	175	300
	RSD	39%	93%		125%	100%		22%	12%
	n	10	10	10	10	10	10	10	10
AD04									
	Avg.	1000	600	0	475	275	0	575	2900
	s.d.	525	550	0	300	250	0	150	275
	RSD	53%	92%		63%	91%		26%	9%
	n	10	10	10	10	10	10	10	10
AD05									
	Avg.	1150	200	0	425	225	0	500	2500
	s.d.	500	250	0	225	225	0	475	575
	RSD	43%	125%		53%	100%		95%	23%
	n	10	10	10	10	10	10	10	10
AD06									
	Avg.	1400	300	50	0	25	0	575	2350
	s.d.	400	425	100	0	75	0	375	575
	RSD	29%	142%	200%		300%		65%	24%
	n	10	10	10	10	10	10	10	10
AD07									
	Avg.	400	10	25	350	350	150	393	1650
	s.d.	20	25	50	200	375	350	287	300
	RSD	50%	250%	200%	57%	107%	233%	73%	18%
	n	10	10	10	10	10	10	10	10
AD08									
	Avg.	350	100	25	225	200	0	875	2025
	s.d.	275	125%	50	150	200	0	575	600
	RSD	79%	125%	200%	67%	100%		66%	30%
	n	10	10	10	10	10	10	10	10

Table 3.4.: Volumes of fluids (mL) consumed in each category and location.

Subject	Category Location	Home	Cold Work	Other	Home	Hot Work	Other	Other	Total
AD09									
	Avg	525	275	175	425	150	125	1350	2925
	s.d.	400	350	375	475	200	150	350	625
	RSD	76%	127%	214%	112%	133%	120%	26%	21%
	n	10	10	10	10	10	10	10	10
AD10									
	Avg.	1550	175	0	450	200	0	1225	3500
	s.d	275	475	0	150	225	0	1075	925
	RSD	18%	271%		33%	113%		88%	26%
	n	7	7	7	5	7	7	7	7
	Overall avg	1045	201	68	245	175	35	939	2705
	Avg. *s.d.*	458	253	113	163	188	63	449	584

Table 3.4. (continued): Volumes of fluids (mL) consumed in each category and location.

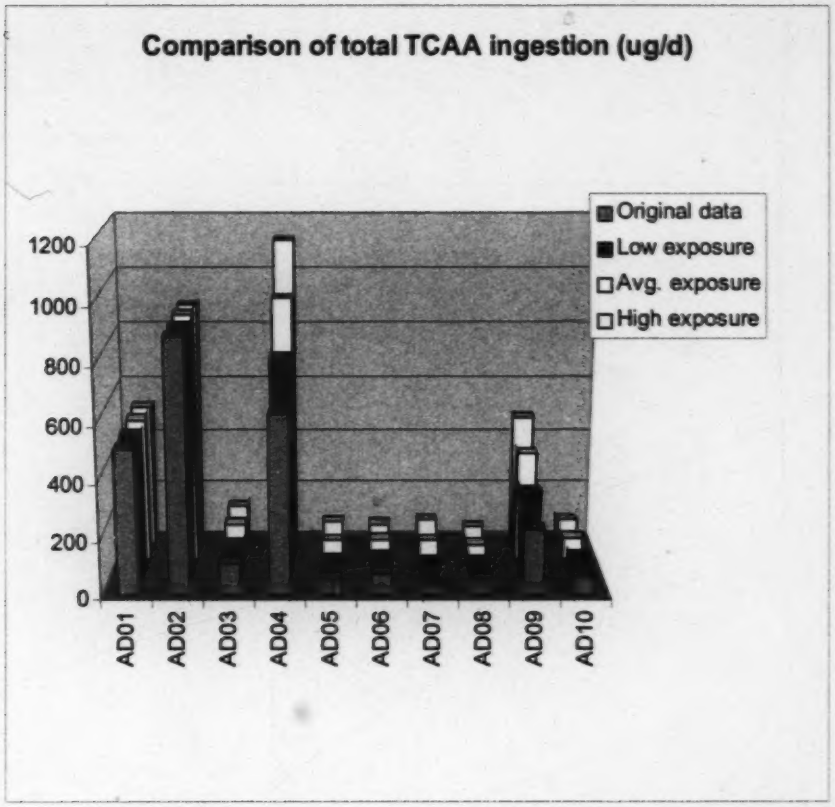

Figure 3.1: Graphical depiction of the increases in total *TCAA-in* when TCAA increases due to boiled water ingestion are applied. The increases are derived from the observed increases in TCAA concentration when coffee is prepared (Chapter 2).

3.4.2 The impact of beverage data on calculated ingestion and excretion values

3.4.2.1 Correlation between measured and calculated TCAA excretion

The Adelaide trial assessed the correlation of TCAA ingestion with TCAA excretion in order to determine the reliability of using TCAA as a biomarker of exposure to DBPs. The correlation between measured TCAA excretion and calculated upper bound excretion (based on TCAA ingestion and applying a TCAA half life calculation to account for the residual TCAA left over from previous days' ingestion) was assessed for the participants. The excretion half life of TCAA refers to the amount of time that it takes for TCAA excretion to decline to 50% of a previously established steady state level of TCAA excretion. The half-life measured in the experiments does not address how TCAA is eliminated (by degradation or by excretion), it only measures the time taken for excretion to decline from an estimated steady state value. Froese et al. (2002) note that, by definition, even after two half lives have passed since TCAA exposure is stopped, up to 25% of the total TCAA from the prior exposure will contribute towards excretion on the day of measurement. Therefore it is imperative to apply a half life calculation to account for TCAA excretion contributions from TCAA ingestion that occurred on previous days. Based on this theory, a maximum potential urinary excretion of TCAA was calculated using a running average of TCAA ingestion that was adjusted according to exponential die-off of the compound in the body. The calculation used was that derived in Froese et al. (2002):

$TCAA_{ex(c)} = 1/m \sum TCAA_{in} e^{-k(t + 0.333)}$

where:

$TCAA_{ex(c)}$ is the estimated maximum potential urinary excretion of TCAA (μg/d)

$TCAA_{in}$ is the calculated urinary ingestion of TCAA (μg/d) for day m

$k = 0.693 / t_{1/2}$

$t_{1/2}$ is the mean half life taken for all those subjects whose half life was measured. In the case of our study, the mean half life of 3d was applied to all calculations.

t in days is incremented from 0 to m-1

m is the nearest integer value to 2 times the individual $t_{1/2}$

Using the above calculation to derive a calculated upper bound excretion level, the calculated value was compared graphically to the measured value for each participant. The basis behind performing this comparison is to determine whether calculated excretion amounts (based on ingestion) correlate well with measured excretion rates. High correlation between calculated excretion (based on ingestion) and measured excretion suggests that the two measures actually do relate to one another. In terms of exposure assessment, this finding would prove to be valuable, since it implies that calculated ingestion is close to the true value, which increases confidence in assessing human exposure to TCAA. The Adelaide study found that there was good correlation for a couple of the participants (namely participants two and six). By comparing the original calculated values for TCAA excretion (based on ingestion) with the calculated values using the mean ± 1 *s.d.* values for TCAA increase in boiled

beverages, I am able to assess whether the reported increases actually affect the ingestion estimates.

Since the TCAA concentrations increase when our beverage data was applied, the correlation between measured and calculated excretion was assessed for each exposure level and compared to the original data presented in the Adelaide trial study. Table 3.2 illustrates the line equations and R^2 values for the participants at each exposure level. It should be noted that participants 2 and 6 were not used, since participant two reported no ingestion of boiled beverages, and participant six ingested such a small amount that the impact would be negligible. All participants that ingested any level of boiled beverages were assessed in this investigation. This was performed to illustrate the fact that ingestion exposure will vary among individuals based on the amount of boiled beverage ingestion. It is expected that the greatest impacts will be noted for those individuals who ingested an appreciable amount of boiled beverages. Large proportions of boiled beverage consumption are expected to affect the original data, while minimal boiled beverage consumption (as a proportion of overall consumption) should present minimal effects. Table 3.5 shows the ratios of fluid types consumed compared to total fluid consumption.

Subject	Category Location	Home/ Total	Cold Work/ Total	Other/ Total	Home/ Total	Hot Work/ Total	Other/ Total	Other
AD01								
	Avg.	31%	0%	13%	0%	0%	2%	54%
	s.d.	18%	0%	17%	0%	0%	4%	23%
AD02								
	Avg.	61%	0%	0%	0%	0%	0%	39%
	s.d.	30%	0%	0%	0%	0%	0%	8%
AD03								
	Avg.	38%	14%	0%	4%	13%	0%	32%
	s.d.	15%	13%	0%	5%	13%	0%	7%
AD04								
	Avg.	34%	21%	0%	16%	9%	0%	20%
	s.d.	18%	19%	0%	10%	9%	0%	5%
AD05								
	Avg.	46%	8%	0%	17%	9%	0%	20%
	s.d.	20%	10%	0%	9%	9%	0%	19%
AD06								
	Avg.	60%	13%	2%	0%	1%	0%	24%
	s.d.	17%	18%	4%	0%	3%	0%	16%
AD07								
	Avg.	24%	1%	2%	21%	21%	9%	24%
	s.d.	12%	2%	3%	12%	23%	21%	17%
AD08								
	Avg.	17%	5%	1%	11%	10%	0%	43%
	s.d.	14%	6%	2%	7%	10%	0%	28%
AD09								
	Avg.	18%	9%	6%	15%	5%	4%	46%
	s.d.	14%	12%	13%	16%	7%	5%	12%
AD10								
	Avg.	44%	5%	0%	13%	6%	0%	35%
	s.d.	8%	14%	0%	4%	6%	0%	31%
	Overall Avg.	37%	8%	2%	10%	7%	2%	34%
	Avg. *s.d.*	17%	9%	4%	6%	8%	3%	17%

Table 3.5: Ratios of fluid types consumed compared to total fluid consumption. (Froese et al., 2002).

Of the eight volunteers analyzed, four illustrated negligible changes in the R^2 (AD1: $R^2_{original} = 0.13$, $R^2_{high} = 0.13$; AD2: $R^2_{original} = 0.32$, $R^2_{high} = 0.33$; AD4: $R^2_{original} = 0.88$, $R^2_{high} = 0.88$; AD5: $R^2_{original} = 0.11$, $R^2_{high} = 0.10$) , two showed a decrease (AD7: $R^2_{original} = 0.46$, $R^2_{high} = 0.36$; AD8: $R^2_{original} = 0.19$, $R^2_{high} = 0.13$) , and two gave an increase in the R^2 value (AD9: $R^2_{original} = 0.13$, $R^2_{high} = 0.26$; AD10: $R^2_{original} = 0.70$, $R^2_{high} = 0.73$) . Of those that illustrated negligible changes in correlation, the consumption diary data helped explain the findings for participants one and four. The low proportion of water ingested via boiled beverages by participant 1 was not expected to affect this individual's maximum potential excretion. Therefore, we did not expect the correlation between calculated and measured excretion to be significantly altered. Participant 4 ingested such a large volume of water that the increase in TCAA exposure generated could not markedly affect the line equation or R^2 value. Figures 3.2 and 3.3 illustrate the plots of ingested TCAA versus excreted for the original and new data. Even large changes in calculated excretion did not substantially change the R^2 values for this participant. At maximum increased exposure, we are looking at doubling a *proportion* of this individual's TCAA ingestion. Participant four was already ingesting such a large amount of TCAA that an increase in TCAA ingestion through boiled beverages did not contribute enough to cause a striking effect on measured versus calculated *TCAA-ex*.

Participant	Original	Low	Mean	High
AD1	Y=0.05x+2.9 R^2=0.13	Y=0.05x+2.9 R^2=0.13	Y=0.05x+2.9 R^2=0.13	Y=0.05x+2.9 R^2=0.13
AD3	Y=0.13x+0.90 R^2=0.32	Y=0.13x+0.90 R^2=0.32	Y=0.13x+0.90 R^2=0.33	Y=0.13x+0.90 R^2=0.33
AD4	Y=0.20x+2.8 R^2=0.88	Y=0.16x+2.9 R^2=0.88	Y=0.13x+2.9 R^2=0.88	Y=0.11x+2.9 R^2=0.88
AD5	Y=0.15x+1.8 R^2=0.11	Y=0.12x+1.8 R^2=0.11	Y=0.10x+1.8 R^2=0.10	Y=0.08x+1.8 R^2=0.10
AD7	Y=0.95x+1.3 R^2=0.46	Y=0.95x+1.3 R^2=0.41	Y=0.95x+1.3 R^2=0.38	Y=0.95x+1.3 R^2=0.36
AD8	Y=0.57x+2.0 R^2=0.19	Y=0.52x+2.1 R^2=0.17	Y=0.47x+2.1 R^2=0.15	Y=0.43x+2.2 R^2=0.13
AD9	Y=0.07x+3.0 R^2=0.12	Y=0.04x+3.0 R^2=0.15	Y=0.03x+3.1 R^2=0.16	Y=0.03x+2.9 R^2=0.26
AD10	Y=1.2x+0.2 R^2=0.70	Y=1.1x+0.1 R^2=0.71	Y=1.0 x+0.02 R^2=0.72	Y=0.91x+0.06 R^2=0.73

Table 3.6: The line equations and R^2 values for measured versus calculated *TCAA-in* for all of the participants studied. The original, low, mean, and high exposure values are given.

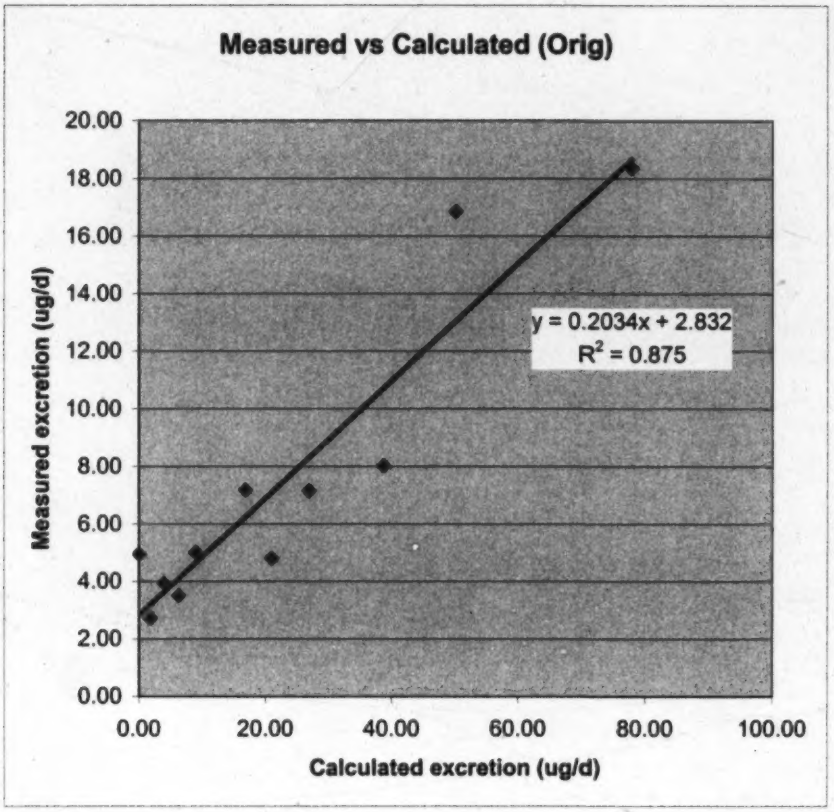

Figure 3.2: Original plot of measured versus calculated TCAA excretion at the originally used exposure level for AD4.

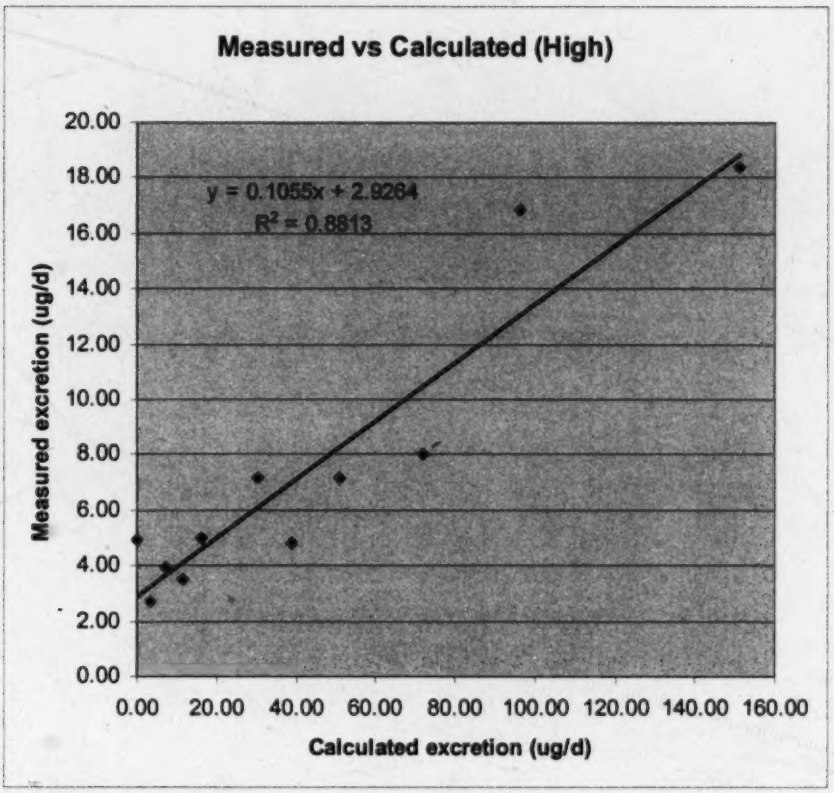

Figure 3.3: Plot of measured versus calculated TCAA excretion at the high exposure level for AD4.

A number of observations are presented for the three participants who illustrated decreases in the R^2 values when measured *TCAA-ex* was compared to calculated *TCAA-ex*. Participant five showed a decrease in the R^2 value from 0.11 to 0.10, which is insignificant. It should be noted that there already was extremely poor correlation to begin with, and the application of the boiled beverage data did not cause a marked impact. This finding is difficult to evaluate, as this volunteer ingested 26% of water through boiled beverages that could be measured (i.e. not commercially prepared). We would have expected a greater impact on the data than what was observed. When the beverage data was applied, subject 8 showed a decrease in the R^2 value from 0.19 to 0.13. However, the low level of correlation in the original data suggests that recalculated data may not be reliable to draw conclusions from. Another participant who showed a decrease in correlation was AD7 (an R^2 decrease from 0.46 to 0.36). In this case, the original correlation was marginally acceptable, and the decrease of 0.10 (a 25% decrease) was quite substantial. It should be noted that this individual ingested a large proportion of TCAA through boiled beverages (approximately 53%). This implies that any changes to the presumed level of TCAA would present significant changes to the calculated TCAA excretion rates, since TCAA ingestion was altered only in the boiled beverage category. It was expected that the R^2 value would be notably affected, since a large proportion of this participant's TCAA ingestion changed when ingestion due to boiled beverage consumption was increased. It must be noted that this individual's relative boiled beverage consumption and total TCAA intake amount was different than the rest of

the study volunteers. Conclusions should not be drawn from one participant who has a different water intake profile than all other participants. This individual's exposure is not representative of the rest of the study population.

Of the participants who illustrated an increase in correlation, one showed a negligible change in the value. The R^2 for AD3 only increased from 0.32 to 0.33. This subject ingested a small proportion of TCAA through boiled beverage consumption, therefore the increased exposure estimate was not expected to cause a profound change. AD9 showed a marked increase in correlation when we applied the increase in TCAA exposure due to boiled beverage ingestion. The R^2 value increased from 0.12 to 0.26. This individual consumed a fair proportion of TCAA from boiled beverages (approximately 31%), therefore changes in the TCAA exposure level from boiled beverages was expected to play a significant role when comparing measured and calculated excretion for this individual. Finally, participant ten showed an increase in the correlation going from $R^2 = 0.70$ (original) to $R^2 = 0.73$ (high exposure). The importance of this data is that the slope of the line was close to one ($m = 0.91$), which suggests that measured and calculated values were also close to one another. The slope near one, and the correlation > 0.70 suggest that this individual's measured and calculated values are reasonably close to one another. The fact that applying the beverage data to the ingestion diaries caused an increase in correlation implies that the additional data is improving our estimate of TCAA exposure. Figure 3.4 and 3.5

illustrate the graph of measured versus calculated excretion rates for the original data and high exposure data (respectively) for subject 10.

When investigating analysis of the correlation between calculated *TCAA-ex* and measured *TCAA-ex*, there is not a clear trend when the beverage data is applied. Some of the correlations improved, some essentially stayed the same, and some got worse. It is important to note that the measure of correlation used, the R^2 value, is essentially a measure of how closely the plotted points fit around the trend line. Chapter two indicates wide variation in TCAA increase for separate coffee samples, let alone variation between different types of heated beverages (i.e. coffee and tea). The wide range in TCAA change from the different heated beverages makes it difficult to assess a clear effect due to hot beverage ingestion (since we do not have specific information regarding the type of hot beverage ingested or the method of preparation). I can not give a reliable estimate of how hot beverage ingestion affects TCAA exposure on any given day, therefore there is uncertainty in the effect of heated beverage consumption for any given point on a plot of measured versus calculated TCAA-*ex*. True effect on correlation cannot be validated without knowing exactly what type of beverage was ingested and how that beverage was prepared, since this affects the extent of TCAA concentration increase.

All in all, we did not see strong correlation between measured and calculated TCAA excretion amounts. The only thing that should be noted is that applying beverage

data *changed* the correlations for most of the individuals. It cannot be stated that applying the beverage data improved the correlation, but it did affect the correlations. With this in mind, applying beverage data is important when trying to validate TCAA as a biomarker, since it clearly makes an impact on the calculated ingestion and excretion amounts.

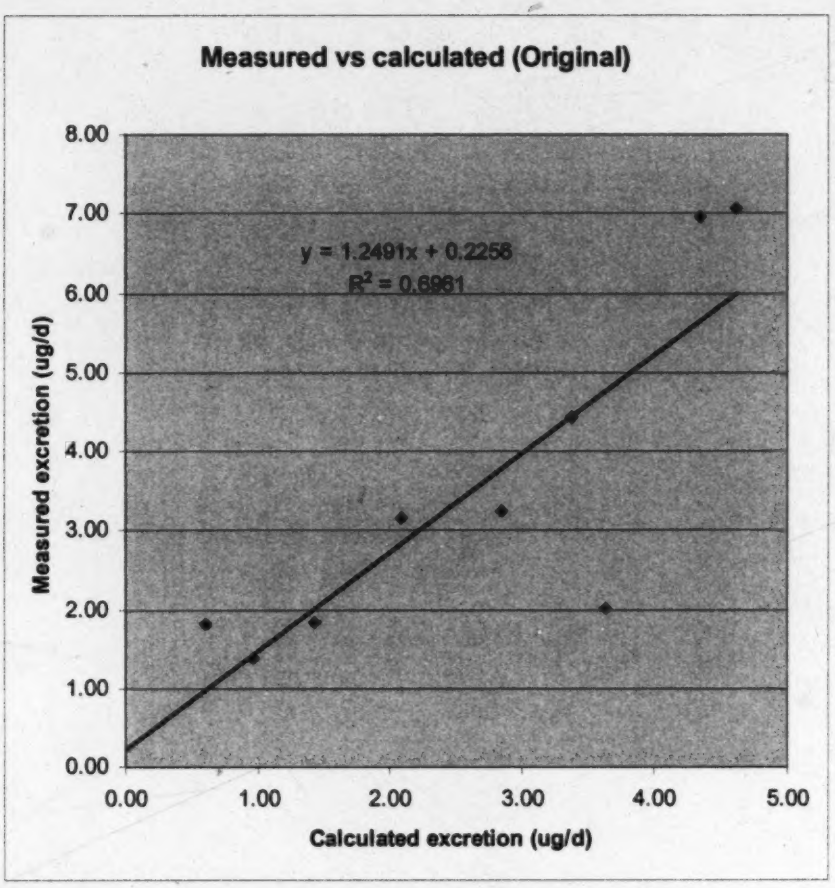

Figure 3.4 Graph of measured versus calculated TCAA excretion rates for participant 10 of the Adelaide pilot study. This data shows the original multiplier of 0.65 used to calculate amount of available TCAA in boiled beverages.

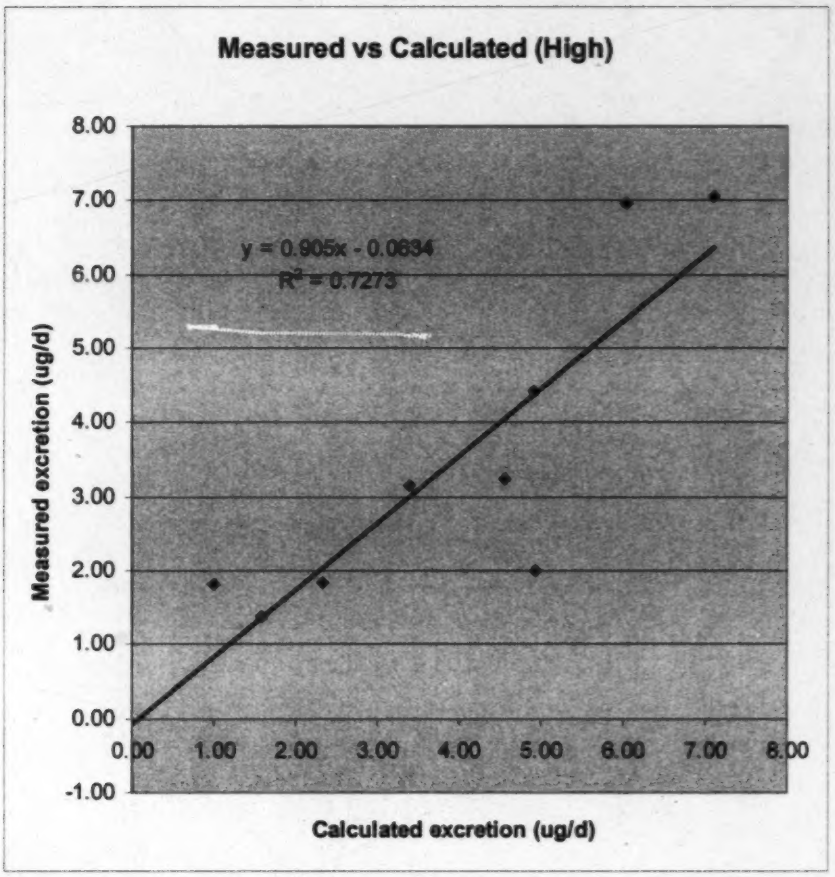

Figure 3.5 Graph of measured versus calculated TCAA excretion rates for participant 10 of the Adelaide pilot study. The calculated excretion rates are based on using a multiplier of 2.86 for TCAA level in boiled beverages, based on the high end of TCAA increase (186%) when coffee was prepared (As described in Chapter 2).

3.4.2.2 Comparing ratios of TCAA-ex to TCAA-in

The ratios of *TCAA-ex* to *TCAA-in* were affected by the applied beverage data, especially in those cases where boiled beverage consumption was substantial or the original TCAA ingestion was low. Table 3.3 indicates the ratios obtained for all participants at all exposure levels. The *TCAA-in* was calculated using Equation 1, applying a mean half-life of 3 days (obtained from averaging the half lives of participants AD1, AD2, and AD6) to calculate a contribution to ingestion for the previous three days (Froese et al., 2002). The results show that the ratios can be greatly affected by the increase in calculated ingestion when beverage data is applied. The presumption by Froese et al. (2002) that participants with low *TCAA-in* may be influenced by small contributions of TCAA from other sources may be correct, as it is clear that some of these ratios are significantly affected by applying beverage data. The overall inter-individual variability in these results appears to be reduced by applying the beverage data. In the original study, the ratios ranged from 0.17 to 1.62; using a single average half life calculation, my values for the original data ranged from 0.14 to 1.8. The highest ratio was obtained from AD7, who also ingested the highest proportion of hot beverages compared to overall water consumption. Applying TCAA concentrations due to beverage consumption, the ratios ranged from 0.14 to 0.83, with the exception being AD8, who was not really affected by applying the beverage data. Using my half-life calculation, AD8 went from a *TCAA-ex / TCAA-in* ratio of 1.6 to a ratio of 1.5. AD8 consumed 43% of his water through

"other" sources, meaning that we have a very large amount of uncertainty for *TCAA-in*. As a result, any inferences regarding *TCAA-in* for this participant are not very reliable. The participants whose ratios were reduced the most were those who ingested a large volume of boiled beverages (AD04, AD05, AD07, AD09, AD10). Seeing a decrease in the overall range of *TCAA-ex / TCAA-in* suggests that the beverage data is reducing some uncertainty with regard to our estimates of excretion and ingestion.

In addition, the ratios of *TCAA-ex* to *TCAA-in* are below 1.0 (with the exception of participant eight). Having most of the ratios below 1.0 makes more logical sense than seeing a number of them above 1.0. Confidence in extrapolation of *TCAA-ex* from first morning urine (FMU) samples is increased. The ratios between the two values should be 1.0 or less, any values above 1.0 must be attributed to errors in extrapolation from FMU or errors in estimation of *TCAA-in*. With most of the ratios below 1.0, the data makes more sense and, presumably, is ultimately more reliable.

Participant	TCAA-ex / TCAA-in		
	Mean	Standard Deviation	RSD%
AD1			
Original	0.14	0.1	71
Low	0.14	0.1	71
Average	0.14	0.1	71
High	0.14	0.1	71
AD2			
Original	0.46	0.41	88
AD3			
Original	0.31	0.15	48
Low	0.29	0.13	46
Average	0.27	0.13	48
High	0.25	0.12	48
AD4			
Original	0.29	0.08	29
Low	0.22	0.07	31
Average	0.18	0.06	30
High	0.15	0.05	33
AD5			
Original	1.0	0.63	62
Low	0.81	0.49	60
Average	0.67	0.41	61
High	0.56	0.32	57
AD6			
Original	1.45	0.95	65
AD7			
Original	1.8	0.54	31
Low	1.1	0.36	32
Average	0.83	0.26	31
High	0.65	0.21	32
AD8			
Original	1.6	1.0	65
Low	1.5	1.0	67
Average	1.5	1.0	67
High	1.5	1.0	67

Table 3.7.: Ratio of *TCAA-ex* to *TCAA*-in using original data, as well as values for low, average, and high TCAA ingestion (using the values obtained from coffee analysis) are provided. TCAA excretion (assuming 100% excretion of ingested), was calculated according to Equation 1, using $t_{1/2}$ = 3 d.

Participant	TCAA-ex / TCAA-in		
	Mean	Standard Deviation	RSD%
AD9			
Original	0.29	0.15	52
Low	0.20	0.12	60
Average	0.15	0.09	60
High	0.13	0.08	62
AD10			
Original	1.2	0.42	34
Low	1.1	0.36	34
Average	0.94	0.32	34
High	0.83	0.29	35

Table 3.7. (Continued).

3.4.2.3 Effects on Temporal Variability

The average value of *TCAA-in* for all participants over 10 days of normal tap water ingestion did not change substantially. As is seen in Table 3.8, the original value was 22 μg/d with an *s.d.* of 9.0 μg/d and a relative standard deviation of 41%, while the highest ingestion level (corresponding to 1 *s.d.* higher than the average increase in TCAA concentration during coffee preparation) of *TCAA-in* showed an average of 30 μg/d with an *s.d.* of 12 μg/d and an RSD of 43%. Considering the fact that some of the participants reported a substantial increase in *TCAA-in* when the beverage data was applied, these results require some explanation. AD2 was the individual who ingested large amounts of TCAA but did not drink any boiled beverages, therefore this individual contributed a lot to the overall average *TCAA-in*, but was not affected by applying the beverage data. The substantial influence on the overall average *TCAA-in* by this individual would prevent us from observing large changes in the overall *TCAA-in* for the group of ten. Repeating the same exercise excluding AD2 changed the average relative increase observed. The observed change from 22 μg/d to 30 μg/d gives a relative increase of 27% (when AD2 is included). The data (given in Table 3.9) excluding AD2 shows an original value of 16 μg/d rising to a maximum of 25 μg/d. This gives a relative increase of 36%. This finding illustrates how the exposure patterns of different individuals can affect the estimates of TCAA-*in* when beverage ingestion is applied.

Table 3.10. also provides the average, standard deviation, and RSD values for all four ingestion levels for each of the ten days for which regular water ingestion was recorded. These data were used to observe the daily differences for standard deviation and RSD when all four exposure groups were compared for all the participants. The standard deviation ranged from 1.3 to 8.3 μg/d, and the relative standard deviation ranged from 7.1 to 21%. These differences are solely attributed to the differences in beverage consumption from day to day. There is a good deal of inter- and intra-individual variability in beverage consumption from day to day, which will ultimately affect *TCAA-in* variability.

	TCAA Exposure Category			
	Original	**Low**	**Mean**	**High**
Average *TCAA-in* (μg/d)	22	24	27	30
Standard Deviation	9.0	10	11	12
RSD%	41	41	42	43

Table 3.8.: Overall average, standard deviation, and RSD% (for all participants) from calculated daily ingestion of TCAA for all four exposure categories.

	TCAA Exposure Category			
	Original	**Low**	**Mean**	**High**
Average *TCAA-in* (μg/d)	16	19	22	25
Standard Deviation	6.0	8.0	10	11
RSD%	41	42	42	43

Table 3.9: Overall average, standard deviation, and RSD% from calculated daily ingestion of TCAA over all four exposure categories, excluding participant two who ingested no boiled beverages.

Ingestion Level	*TCAA-in (μg/d)*										
	30-Jan	31-Jan	1-Feb	5-Feb	6-Feb	7-Feb	8-Feb	9-Feb	10-Feb	12-Feb	13-Feb
Original	32 ± 43	20 ± 22	27 ± 39	38 ± 52	22 ± 26	23 ± 30	29 ± 45	13 ± 17	10± 13	14 ± 19	13 ± 13
Low	35 ± 49	22 ± 23	29 ± 40	44 ± 58	26 ± 32	24 ± 31	30± 45	14 ± 17	11 ± 13	16 ± 21	15 ± 16
Mean	39 ± 57	24 ± 24	31 ± 41	51 ± 67	31 ± 39	26 ± 31	32 ± 45	16 ± 16	12 ± 13	18 ± 23	18 ± 19
High	43 ± 66	26 ± 26	33 ± 43	57 ± 77	36 ± 47	27 ± 32	34 ± 46	17 ± 17	13 ± 14	20 ± 25	20 ± 23
Average	37	23	3.0x10	48	29	25	31	15	12	17	17
s.d.	4.8	2.6	2.6	8.3	6.1	1.8	2.2	1.8	1.3	2.6	3.1
RSD%	13	17	8.6	17	21	7.3	7.1	12	11	15	19

Table 3.10: Average *TCAA-in* values for all participants for each day of reported water ingestion. The original calculated values (corresponding to a 35% reduction in TCAA for boiled beverages), as well as the mean ± 1 s.d. values for TCAA increase in boiled beverages are included. Each exposure level is compared using standard deviation and RSD% for each study day.

3.5 Conclusions

The extensive ingestion diaries completed by the study subjects in the Adelaide pilot trial allowed us to observe the effect of using the noted increases to TCAA concentration post beverage preparation to re-evaluate TCAA exposure in the study participants. The TCAA exposure was quite varied across the study population, because of the significant variation in amount and type of water ingestion. This variation resulted in large discrepancy in estimated TCAA exposure increases, both numerically and percentage-wise, for the study subjects when the increase in TCAA concentration was accounted for in hot beverages. It should be noted, however, that some of the subjects experienced profound increases in the estimate of TCAA being ingested. As shown in Table 3.1, AD4 had an original overall TCAA ingestion of 630 μg, and this level jumped to 1100 μg for the highest exposure level. It is clear that TCAA ingestion estimates could be greatly affected by beverage ingestion. It should be noted, however, that that the effects noted are based on a paper exercise whereby original ingestion exposure estimates were manipulated with experimental data obtained for TCAA production in coffee making. However, the observed increases demonstrate the scale to which we may be underestimating true ingestion exposure.

Conversely, estimates for some subjects illustrated profound relative increases, but the numeric value of TCAA was quite minimal. An individual who ingests little tap

water, but a large proportion of which is from hot beverages, will display large increases in exposure when observed as a percentage, but the overall level of TCAA ingested is still low. For example, AD7 showed an increase in estimated maximum TCAA exposure of 180%, but the total increase in TCAA exposure at this level would still only be 56μg (Table 3.1). This value, compared to the 1100μg ingested by AD4, is rather small. Therefore, TCAA exposure from hot beverages may have little effect on TCAA exposure estimates when there is such a substantial disparity in tap water ingestion across the study population. What these data do provide is the rationale that TCAA ingestion *can* be greatly affected by what an individual ingests. Determining exposure to TCAA by simply measuring drinking water and applying a general TCAA exposure level based on drinking water analysis provides a huge margin of error in the estimation of ingestion. It is necessary to obtain information about all ingestion routes in order to obtain a more comprehensive, and ultimately more accurate, measure of TCAA exposure.

Correlation between calculated *TCAA-ex* (based on TCAA ingestion and application of TCAA half-life in the body) with measured *TCAA-ex* did not provide evidence for a definitive conclusion regarding the effect of applying the beverage data to TCAA ingestion. What these data did show is that applying increases on exposure based on beverage consumption affects the correlation with measured *TCAA-ex*. Since there is a noted effect, it is important that we understand the fact that applying beverage consumption when calculating TCAA exposure will affect the exposure information.

In terms of validating TCAA as a biomarker of exposure, it is important to note any effects that varying forms of water ingestion will have on TCAA exposure. The results do not preclude the possibility that better exposure estimates are possible, but the available results do not imply a *certain* benefit. Therefore, the evidence serves to reinforce the importance of looking at TCAA ingestion from beverages when trying to determine exposure.

The approach used certainly illustrates shortcomings in previously used methods of TCAA ingestion estimates, but it may not reveal the whole truth either. Difficulties lie in the amount of variation in form of water ingestion and amount of water ingested for each participant. First of all, the controlled experiments performed (Chapter 2) show wide variation that is illustrated in the large standard deviation for relative increase in TCAA during coffee preparation. For this investigation, fixed TCAA increases were applied based on the mean ± 1 standard deviation for replicate experiments investigating TCAA increase during beverage preparation. Clearly there is much wider variation than this. TCAA increases due to beverage preparation may vary greatly for each participant based on the way they prepare coffee and the type of coffee prepared. In addition, coffee is not the only hot beverage ingested, but only coffee data was used in this investigation. Tea showed similar experimental results when compared to coffee (Chapter 2), but again there are a multitude of different tea brands and methods of preparation. Furthermore, there are other hot beverages that were not addressed (hot chocolate, hot lemonade, etc.). The study could not take into

account the wide variety of hot beverages ingested, and thus could not address the fact that individual TCAA increases might vary widely for each participant. Another drawback is the fact that a large amount of water ingested was classified as "other", which referred to sources that were commercially prepared and could not be analyzed. It was noted that individuals who ingest large proportions of boiled beverages will show greater effects when the increases due to hot beverage preparation were applied. This notion holds true for those who ingest large proportions of "other" beverages. The proportion of "other" beverages consumed ranges from 20% to 54% (Table 3.4). All participants ingestion will be affected, but we have no way to speculate what this ingestion contributes to TCAA exposure. We are looking at overall *TCAA-ex*, but our *TCAA-in* estimate overlooks at least 20% of fluid ingested. My data has shown that variation in type of fluid consumption can affect TCAA ingestion, therefore the "other" category contributes a lot of uncertainty with respect to *TCAA-in*. As a result, it is difficult to make a conclusion on reliability of TCAA ingestion estimates.

Applying the beverage data served to reduce the range of *TCAA-ex / TCAA-in* values, and the tighter set of values implies increased precision in the estimates. Since we are observing a ratio in which two values are compared, the increased precision suggests that *TCAA-ex* and *TCAA-in* values are closer to one another. While it is possible that this evidence suggests *TCAA-ex* and *TCAA-in* are similarly inaccurate rather than similarly accurate, it is still important to understand the value of the observed

relationship. Theoretically, there should be a good relationship between the TCAA that goes in to the body and the TCAA that comes out. Observing a smaller range of values when comparing *TCAA-ex* to *TCAA-in* (using hot beverage ingestion data) certainly implies a better relationship between the two values. This provides evidence that the beverage data *may* be improving our estimate of *TCAA-in*, therefore it should not be ignored. In addition, the fact that the relationship between *TCAA-ex* and *TCAA-in* changes when beverage data is applied suggests that the beverage information is affecting the ingestion data, and therefore should be accounted for.

Overall, it is difficult to say whether the beverage data is in fact improving our estimate of *TCAA-in*. There are some promising results that suggest the beverage data reduces some of the uncertainty in TCAA ingestion present in this study. Conclusive results are hindered by the small sample size, presence of individuals who drank little or no boiled beverages, and the large volume of "other" beverages ingested for which there is no available TCAA ingestion data. However, the applied beverage data does show that *TCAA-in* is affected, which means that ingestion and exposure estimates should include a reliable measure of TCAA contribution from beverages. In addition, it was shown that inter-individual variability in TCAA ingestion is affected by boiled beverage consumption. Differences in type of water consumed should be addressed when assessing DBP intake, as it clearly varies from person to person.

In terms of developing an accurate biomarker of exposure to DBPs, it is important to look at how ingestion can be affected when a person consumes boiled beverages. Chapter two indicated that the preparation of boiled beverages serves to increase TCAA and DCAA concentrations, therefore an individual who consumes boiled beverages will ingest more TCAA and DCAA than drinking tap water. Obtaining an accurate estimation of TCAA-*in* is paramount in the development of using TCAA as a biomarker of exposure to DBPs. We need to know how much TCAA is actually coming into the body to identify how urinary TCAA reflects the total TCAA ingestion.

It should also be noted that our exposure assessment only took into account TCAA ingestion from cold tap water and hot tap water (boiled beverages). There are a number of other routes of exposure, including food ingestion, activities such as swimming, bathing, and washing items that may also be contributing to overall exposure. Therefore, applying the boiled beverage data to the cold tap water ingestion data has helped to formulate a more complete picture of exposure, there are still many unknowns that prevent us from understanding total exposure to DBPs. This work has shown us that other factors of ingestion certainly do affect exposure, therefore it is necessary to investigate other sources of exposure than cold tap water alone.

3.6 References

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Chapter 4: Synthesis and Overall Conclusions

4.1. Conclusions

Comparative analysis of tap water samples and beverage samples prepared with the same tap water suggest that there are notable increases in TCAA and DCAA concentrations during beverage preparation. Theoretically, this observation should not be considered surprising. As mentioned in earlier chapters, the basis of DBP formation lies in the reaction of residual chlorine in waters with natural organic matter that are also present. Any tap water source will have an appreciable level of residual chlorine present in order to reduce risks associated with pathogens that may come into contact with the water on the way to the consumer. Beverages are essentially a composite mixture of many different organic compounds. Therefore, introducing a beverage mixture provides the framework for the formation of DBPs in water. The results of SPME GC-ECD analysis of samples provided support for this hypothesis. As a result, it seems reasonable to analyze DBP levels in beverages when trying to obtain a comprehensive estimate of exposure in an individual, since the DBP concentrations in a beverage may be significantly different than that of tap water.

The evidence of chemical reactions taking place that lead to increases in TCAA and DCAA concentrations imply that the levels of other DBPs may be increasing as well. The basis for formation is essentially the same for many of the DBPs (i.e. oxidative fragmentation and chlorination of dissolved organic molecules). In addition, with the

limited data available, it is impossible to say whether compounds not yet identified are forming during the beverage preparation process. Furthermore, the differences in chemical composition of specific beverage mixtures, combined with the varying conditions under which a given beverage is prepared, suggests that the DBP concentrations in each beverage may vary. With a substantial number of unknown factors, it is imperative that we delve deeper into understanding DBP formation beyond simply looking at natural organic matter in water. Development of a reliable biomarker of exposure to DBPs requires an understanding of how the chosen biomarker reflects exposure to other DBPs. The beverage research has shown us that the DBP concentrations may be changing during beverage preparation. This is an important factor to take into account when trying to develop a biomarker.

Exposure studies that apply DBP levels found in beverages will provide a more comprehensive estimate of the exposure profile of a given individual. The noted difference in DBP levels between beverages and water samples supports this fact. Any reliable conclusions drawn from human exposure to DBPs, be it identification of a biomarker or observed human health effects, are contingent on an accurate estimation of exposure. While it is clear that daily exposure to many of these compounds is estimated to be very small (in the low μg/L) and that doubling these small numbers (based on beverage analysis) still leads to very low exposure values, the increases should not be ignored. For those who ingest a large amount of water through beverages, acute and chronic exposure to DBPs stands to be markedly higher

than that of water alone. The lack of physiologically-based pharmacokinetic models for humans (with regard to TCAA) suggests that we do not know enough about these compounds to conclude that exposures are not creating human health problems. We lack accurate exposure estimates for TCAA and other DBPs, and we cannot reliably associate DBP exposure to any known health effects. All we can say is, based on toxicologic studies on animals and some hypothesis-generating epidemiologic studies, it is possible that DBP exposure can cause adverse health effects. The epidemiologic studies performed are deemed hypothesis-generating because they have not created definitive links between DBP exposure and disease; they simply suggested an association.

Exploring and understanding the true level of exposure serves to help elucidate the extent of risk. The intention is not to say that we are at greater risk for certain deleterious health effects due to the increased level of exposure. We are trying to identify the true extent of exposure in order to identify what, if any, risks are presented to the general public. As mentioned earlier, we have studies that have made suggestions, now we need accurate exposure estimates to help us determine if there are definitive links between DBP exposure and disease. In addition, we want to identify the extent of these risks, but this information will not come without a reliable measure of exposure and comprehensive epidemiological studies with regard to human health effects. This work was not intended to suggest that we are at some higher level of *risk* because TCAA exposure might be underestimated. The work was

done to propose the idea that accurate TCAA exposure estimates should take into account all sources of water ingestion. Weisel et al. (1999), Bader (2001), and Froese et al. (2002) have illustrated the promise of TCAA as a biomarker of DBP exposure. My work was done to facilitate the use of TCAA as a biomarker by improving exposure estimates and illustrating potential problems with using this compound as a biomarker. A valid biomarker to DBP exposure will help elucidate exposure to DBPs, information that will be immensely useful in assessing the impact of DBPs on human health.

Presently, due to lack of knowledge with regard to TCAA ingestion exposure and how that reflects exposure to other DBPs, all we can say is that urinary TCAA is a biomarker of exposure to TCAA. My work has shown that TCAA and DCAA concentrations are increasing during beverage preparation, which raises questions with regard to the fate of other DBPs. We are unsure if all the DBP concentrations increase, and by how much. It is unclear whether the increases vary significantly based on water or beverage source. In addition, the data presented in Chapter 2 illustrates the changes in relative ratios of DBPs when different beverages and water sources are applied. TCAA and DCAA concentrations increase differently relative to one another depending on the beverage substrate used. Different reaction conditions favor formation of different DBPs, therefore increases in different DBPs are likely to change based on what beverage is being prepared and how the preparation is carried out. Since the nature of humic and fulvic contents in natural waters will affect the

types of DBPs forming, it is reasonable to assume that the same is true for different beverage sources. Furthermore, it is still unknown whether the more volatile THMs are decreasing during hot beverage preparation (as expected), or if they are increasing as well. Beverage ingestion not only affects the amount of TCAA ingestion, but it also may affect the extent of exposure to other DBPs. This needs to be addressed in order to gain an accurate estimate of how TCAA reflects exposure to other DBPs.

Based on the available information, it would be premature for me to say that applying beverage data to consumption surveys is enhancing our accuracy in estimating TCAA ingestion. The small sample size (n=10) for the Adelaide study, coupled with the large amount of uncertainty in what was consumed (based on the large contingent of "other" beverages ingested) makes it difficult to accurately determine the overall impact of beverage consumption on TCAA ingestion. A larger study with very stringent control over fluid ingestion and accurate measures of all types of fluid ingestion would help to clarify the uncertainty in the contribution of beverages to overall DBP ingestion. Since DBP ingestion is so multiroute, it is difficult to determine how much of an impact the increased exposure through beverages causes without knowing how much beverage consumption applies to overall water consumption, including food preparation, chloral hydrate exposure, etc.

As noted in Chapter 3, there is an immense amount of variation in exposure estimates for each participant in the study. This variation makes it difficult to manipulate the

data and generate valid conclusions from the new data. First of all, Chapter 2 illustrated the wide degree of variation in TCAA relative increases for coffee samples that were prepared *in the same manner*. It is possible that the variance could be worse when you take into account different people preparing coffee in different ways with different coffees. For the feasibility of this study, fixed increases were applied to boiled beverage ingestion estimates. This does not take into account the variation that is certainly present. With this in mind, it is difficult to gain any accurate conclusions with respect to increases in exposure. Again, all the data says is that applying the increases in TCAA exposure due to boiled beverages does change an individual's exposure. This problem could be addressed in a future exposure study by extensive analysis of one method of coffee preparation and ensuring all participants in the study follow this method.

The Adelaide pilot trial creates uncertainty based on the large proportion of fluids ingested that are not accounted for. The "other" category ranged from 20% to 54% of all fluids ingested for the participants in the study (shown in Table 3.4). This is a substantial amount of water ingestion that is not understood with respect to its contribution to overall TCAA ingestion. Uncertainty here also makes it difficult to make any strong conclusions when comparing *TCAA-in* and *TCAA-ex*, both in my re-evaluation of the data and the original study. We cannot definitively state whether exposure estimates are affected positively, negatively, or not at all when beverage consumption is applied if at least 20% of ingestion is not taken into account. This

proportion can potentially cause a major impact on overall TCAA exposure. Further studies should try to eliminate this level of uncertainty to obtain a more definitive estimate of overall exposure.

Furthermore, method development is necessary for the analysis of DBPs in beverages. The SPME method, while proving reliable in the sense that triplicate analyses provided highly reproducible results and the MDLs were quite low, still has inherent drawbacks that makes it difficult to confidently assess DBP concentrations. There is a lack of a reliable internal standard, something that is necessary if we want to quantify DBP concentrations. Different fibers and different absorption and desorption conditions proved optimal for different DBPs, therefore it would be difficult to assess a wide range of DBPs in a sample using one fiber and one method. If we are trying to use TCAA as a biomarker, it is necessary to gain an understanding of how TCAA exposure reflects exposure to other DBPs. Since TCAA and DCAA concentrations were affected to a different extent by beverage preparation (Chapter 2), it is likely that other DBPs would be differentially affected as well. At present, there is not a SPME method that will provide reliable relative ratios of a broad range of DBPs in a beverage sample. The method should be performed in parallel with other proven methods (such as liquid-liquid extraction) in order to validate its accuracy in determining all nine haloacetic acids currently under regulatory control.

This work is intended to shed light on the issue of DBP ingestion in order to come up with a better understanding of exposure to DBPs. The promise of TCAA being a reliable biomarker (Weisel et al., 1999; Froese et al., 2002) meant that we needed an accurate measure of overall TCAA ingestion and an understanding of how this relates to ingestion of other DBPs. While health effects due to DBP exposure have been explored by some researchers, this work was *not* intended to suggest that beverage ingestion leads to increased risk of negative human health impacts. The work has shown that beverage ingestion leads to higher TCAA and DCAA ingestion, but until we have a firm understanding of exposure to DBPs, it is premature to imply that these increases will cause adverse health effects.

Overall, the research shows that beverage ingestion leads to increases in TCAA and DCAA ingestion, and possibly different ingestion profiles of other DBPs compared to straight tap water consumption. The variability in the way water is consumed will lead to increased variability in DBP ingestion. Therefore, an accurate measure of DBP ingestion must take into account the consumption of beverages. The only way to elucidate the overall effect of beverage consumption is to perform a larger, more controlled study that accurately records all routes of water ingestion. Attempting to uncover some of the uncertainties associated with TCAA ingestion exposure has actually served to create more uncertainties that need to be addressed. Ultimately, we need to identify an accurate estimate of TCAA ingestion exposure (taking into

account all routes of ingestion) and understand how TCAA ingestion reflects exposure to other DBPs, which may also be changing during beverage preparation.

4.2. References

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APPENDIX A: RAW DATA

Sample	[TCAA] (μg/L)	Avg [TCAA] (μg/L)	*s.d.*	RSD%	[DCAA] (μg/L)	Avg. [DCAA] (μg/L)	*s.d.*	RSD%
TW1	4.3901				4.9209			
TW2	7.3371	5.3394	1.7308	32.416	15.4402	8.2609	6.2217	75.315
TW3	4.2909				4.4397			
Cof 1	7.4211				26.4103			
Cof 2	6.6114	7.3768	0.7442	10.088	24.4680	26.3123	1.7973	6.831
Cof 3	8.0978				28.0586			
TW1	3.4366				3.5387			
TW2	3.4410	3.5077	0.1194	3.404	4.3201	3.5796	0.7209	20.139
TW3	3.6455				2.8800			
Cof 1	8.8094				27.4765			
Cof 2	9.6251	9.7393	0.9920	10.186	28.5965	29.3636	2.3659	8.056
Cof 3	10.7835				32.0179			
TW1	2.5193				2.8189			
TW2	3.7175	2.7580	0.8652	31.371	3.8416	3.0014	0.7654	25.501
TW3	2.0371				2.3473			
Cof 1	6.8913				33.307			
Cof 2	6.7723	7.7443	1.6259	20.995	32.5835	36.2613	5.7550	15.871
Cof 3	9.6192				42.8934			
TW1	3.6057				2.4911			
TW2	3.4825	3.4809	0.1256	3.608	3.4131	3.0478	0.4899	16.074
TW3	3.3546				3.2393			
Cof 1	6.3708				17.9684			
Cof 2	6.1431	6.8106	0.9657	14.179	18.5257	20.3831	3.7103	18.203
Cof 3	7.9179				24.6553			
TW1	8.4207				8.8007			
TW2	2.7838	4.7547	3.1779	66.837	2.4005	4.9193	3.4108	69.34
TW3	3.0595				3.5566			
Cof 1	6.0118				15.3845			
Cof 2	5.0027	6.0625	1.0860	17.913	11.8707	14.5013	2.3258	16.039
Cof 3	7.1730				16.2848			

Table A1: Results of analysis for tap water samples and coffee samples prepared with the same tap water. The table is divided into separate experiment blocks.

Sample	[TCAA] (μg/L)	Avg. [TCAA] (μg/L)	*s.d.*	RSD%	[DCAA] (μg/L)	Avg. [DCAA] (μg/L)	*s.d.*	RSD%
TW1	1.6995				2.8755			
TW2	1.239	2.6688	2.0905	78.331	2.0865	3.5877	1.9570	54.547
TW3	5.068				5.801			
Cof 1	8.9067				34.801			
Cof 2	7.8758	8.2474	0.5725	6.942	30.1688	31.8075	2.5963	8.163
Cof 3	7.9597				30.4527			
TW1	3.7053				3.1571			
TW2	4.3888	4.8419	1.4185	29.296	2.4049	2.7745	0.3763	13.563
TW3	6.4316				2.7615			
Cof 1	6.5423				19.6777			
Cof 2	6.3642	6.6337	0.3250	4.899	23.0130	22.3680	2.4328	10.876
Cof 3	6.9947				24.4133			

Table A1: Continued

Sample	[TCAA] (μg/L)	Avg. [TCAA] (μg/L)	*s.d.*	RSD%	[DCAA] (μg/L)	Avg. [DCAA] (μg/L)	*s.d.*	RSD%
TW1	2.5193				2.8189			
TW2	3.7175	2.7580	0.8652	31.371	3.8416	3.0014	0.7654	25.501
TW3	2.0371				2.3437			
Tea 1	5.5208				18.5816			
Tea 2	4.9712	5.1839	0.2951	5.693	17.3412	17.8840	0.6345	3.548
Tea 3	5.0598				17.7292			
TW1	3.6057				2.4911			
TW2	3.4825	3.4809	0.1256	3.608	3.4131	3.0478	0.4899	16.074
TW3	3.3546				3.2393			
Tea 1	6.2840				26.8559			
Tea 2	6.3831	6.0702	0.4588	7.558	23.5607	23.8555	2.8644	12.007
Tea 3	5.5436				21.1500			
TW1	3.7053				2.5831			
TW2	4.3888	3.9329	0.3949	10.041	2.6856	2.6493	0.2500	9.436
TW3	3.7045				2.8791			
Tea 1	8.0738				22.5156			
Tea 2	7.4004	7.1568	1.0601	14.812	20.9489	20.4286	2.3900	11.699
Tea 3	5.9961				17.8214			

Table A2: Results of analysis for tap water samples and tea samples prepared with the same tap water. The table is divided into separate experiment blocks.

Sample	[TCAA] (μg/L)	Avg. [TCAA] (μg/L)	*s.d.*	RSD%	[DCAA] (μg/L)	Avg. [DCAA] (μg/L)	*s.d.*	RSD%
TW1	3.6057				2.4911			
TW2	3.4825	3.4809	0.1256	3.608	3.4131	3.0478	0.4899	16.074
TW3	3.3546				3.2393			
IT 1	10.4144				20.2310			
IT 2	3.6201	6.5773	3.4816	52.934	5.4152	12.6048	7.4175	58.847
IT 3	5.6974				12.1682			
TW1	1.6995				2.8755			
TW2	1.2390	2.6688	2.0905	78.331	2.0865	3.5877	1.9570	54.547
TW3	5.068				5.801			
IT 1	4.931				9.7745			
IT 2	5.0157	5.0632	0.1613	3.186	11.5025	11.8191	2.2198	18.781
IT 3	5.243				14.1802			
TW1	2.1280	2.1820	N/A	N/A	1.462	1.4620	N/A	N/A
IT 1	7.2820				10.2585			
IT 2	4.5745	6.6178	1.8053	27.279	4.7755	8.2702	3.0361	36.711
IT 3	7.9970				9.7765			

Table A3: Results of analysis for tap water samples and iced tea samples made with the same water. The separate blocks signify separate experiments performed.

Sample	[TCAA] (μg/L)	Avg. [TCAA] (μg/L)	s.d.	RSD%	[DCAA] (μg/L)	Avg. [DCAA] (μg/L)	s.d.	RSD%
MQ 1	0.3308	0.2988	0.0453	15.16	0.4892	0.5301	0.0578	10.90
MQ 2	0.2667				0.5709			
Cof 1	0.8857				7.2142			
Cof 2	0.9953	1.1809	0.420	35.57	3.8282	8.8315	5.9784	67.69
Cof 3	1.6617				15.4522			
MQ 1	0.1089	N/A	N/A	N/A	0.3329	N/A	N/A	N/A
Cof 1	0.2822	N/A	N/A	N/A	3.2180	N/A	N/A	N/A
MQ 1	0.1467	N/A	N/A	N/A	0.5149	N/A	N/A	N/A
Cof 1	0.5438	N/A	N/A	N/A	5.0844	N/A	N/A	N/A
MQ 1	0.0379	N/A	N/A	N/A	0.2027	N/A	N/A	N/A
Cof 1	0.8117	N/A	N/A	N/A	3.1886	N/A	N/A	N/A
MQ 1	0.1161	0.0905	0.0363	40.11	0.3008	0.3273	0.0375	11.46
MQ 2	0.0648				0.3538			
Cof 1	0.1894				3.3713			
Cof 2	0.2278	0.1625	0.0572	35.2	3.2071	3.8646	0.6755	17.48
Cof 3	0.1329				4.5652			
Cof 4	0.0977				4.3147			
MQ 1	0.0863	N/A	N/A	N/A	0.2027	N/A	N/A	N/A
Tea 1	0.2413				1.7886	N/A	N/A	N/A
Tea 2	0.0970	0.1412	0.0869	61.54	N/A	N/A	N/A	N/A
Tea 3	0.0852				N/A	N/A	N/A	N/A
MQ 1	0.0379	N/A	N/A	N/A	0.2027	N/A	N/A	N/A
Tea 1	0.1452	N/A	N/A	N/A	4.2234	N/A	N/A	N/A

Table A4: Experimental results showing TCAA and DCAA concentrations in Milli-Q water and beverages prepared with the same Milli-Q water.

Sample	[TCAA] (μg/L)	Avg. [TCAA] (μg/L)	s.d.	RSD%	[DCAA] (μg/L)	Avg. [DCAA] (μg/L)	s.d.	RSD%
MQ 1	0.0565	0.0673	0.0152	22.59	0.3008	0.3273	0.0375	11.46
MQ 2	0.0780				0.3538			
IT 1	0.1242				0.9643			
IT 2	0.1181	0.1212	0.0043	3.55	1.4782	1.1056	0.3258	29.47
IT 3	N/A				0.8743			

Table A4: Continued.

Sample	[TCAA] (μg/L)	Avg. [TCAA] (μg/L)	*s.d.*	RSD%	[DCAA] (μg/L)	Avg. [DCAA] (μg/L)	*s.d.*	RSD%
FW 1	1.2313				1.7752			
FW 2	3.0377	2.6020	1.2130	46.62	3.6746	3.2608	1.3280	40.73
FW 3	3.5370				4.3326			
Cof 1	4.4661				13.0570			
Cof 2	2.9814	3.4429	0.8875	25.78	9.3376	10.2356	2.4967	24.39
Cof 3	2.8813				8.3121			
FW 1	2.7857				2.3307			
Cof 1	3.2852				10.0060			
Cof 2	5.3674	4.0191	1.1692	29.09	16.6877	12.1304	3.9498	32.56
Cof 3	3.4047				9.6974			

Table A5: The TCAA and DCAA concentrations found from analyzing water run through a water filtration pitcher and coffee made with the same water.

Spike level	[Chlorine] (mg/L)	[TCAA] (μg/L)	[DCAA] (μg/L)
1	0.05	0.2822	2.8524
2	0.23	0.3144	8.5444
3	1.32	0.5554	7.3325
4	2.73	0.7524	10.8582
5	12.72	2.7865	36.9653
1	0.05	0.6699	2.719
2a	0.37	0.3813	3.5259
2b	0.37	0.3739	3.1326
2c	0.37	0.3653	2.9709
3	1.29	0.998	10.1311
4a	2.62	0.5794	8.5549
4b	2.62	0.6452	11.1213
4c	2.62	0.6173	8.8728
5	11.97	3.189	40.0894
6	134	47.6	351.9974
1	4.25	2.8075	19.1988
2	8.37	3.427	27.5278
3a	17.36	5.8774	53.6736
3b	17.36	5.4699	48.2192
3c	17.36	5.7565	52.3074
1	3.35	4.6459	25.4127
2	9.13	5.4724	33.2296
3a	19.96	8.8106	61.3429
3b	19.96	5.6538	44.4034
3c	19.96	7.9566	58.5367
1	3.95	2.7876	20.0708
2	9.24	3.0893	27.2175
3a	19.86	5.7252	48.1038
3b	19.86	5.6497	46.5414
3c	19.86	5.4552	41.9687

Table A6: TCAA and DCAA concentrations in coffee brewed using water containing different concentrations of residual chlorine.

Sample Level	[Chlorine] (mg/L)	[TCAA] (μg/L)	[DCAA] (μg/L)
1	0.09	0.0565	1.4094
1a	0.09	0.0780	0.4955
2	1.19	1.4622	5.9454
2a	1.19	1.2250	5.2042
3	1.54	1.3145	7.3482
3a	1.54	1.2112	6.3487
4	2.48	4.2735	14.2028
4a	2.48	4.3944	15.6082
5	4.20	9.4430	34.5929
5a	4.20	9.3700	34.5082
1	0.08	0.1242	0.9673
1a	0.08	0.1181	0.4162
2	1.16	0.3917	3.6286
2a	1.16	0.4024	3.6397
3	1.55	0.8653	6.0178
3a	1.55	0.5548	4.4317
4	2.75	1.3113	9.6277
4a	2.75	2.0448	9.8186
5	4.51	2.7457	18.5247
5a	4.51	2.5905	18.9555
1	0.05	N/A	0.8743
1a	0.05	N/A	1.4782
2	17.8	38.5094	114.0655
2a	17.8	32.8155	152.7794
3	129.5	225.9497	427.6920
3a	129.5	223.5662	383.6562

Table A7: The TCAA and DCAA concentrations in iced tea samples prepared with water containing different concentrations of residual chlorine.

Sample	[Chlorine] (mg/L)	[TCAA] (μg/L)	[DCAA] (μg/L)
Spike MQ	2.19	0.5933	1.7843
Spike MQ2	2.19	0.5300	2.4437
Spike MQ3	2.19	0.3234	1.4132

Table A8: TCAA and DCAA concentrations in Milli-Q water samples spiked with Javex bleach (5.25% available chlorine).

Sample	[Residual chlorine] (mg/L)	[TCAA] (μg/L)	[DCAA] (μg/L)
TW	2.30	4.3901	4.9209
TW		7.3371	15.4402
TW		4.2909	4.4397
TW	2.23	3.4366	3.5387
TW		3.4410	4.3201
TW		3.6455	2.8800
TW	2.11	2.5193	2.8189
TW	2.20	3.7175	3.8416
TW	2.23	2.0371	2.3473
TW	2.22	3.6057	2.4911
TW	2.31	3.4825	3.4131
TW		3.3546	3.2393
TW	2.22	8.4207	8.8007
TW		2.7838	2.4005
TW		3.0595	3.5566
TW	2.21	1.6995	2.8755
TW		1.2390	2.0865
TW		5.068	5.801
TW	2.15	3.7053	3.1571
TW		4.3888	2.4049
TW		6.4316	2.7615
Averages	**2.22 ±0.06**	**3.9073±1.7468**	**4.1863±2.9917**

Table A9: Summary of TCAA and DCAA concentrations found in Edmonton tap water. Residual chlorine concentrations are included for some of the samples.

Coffee		Tea		Iced tea	
[TCAA] (μg/L)	[DCAA] (μg/L)	[TCAA] (μg/L)	[DCAA} (μg/L)	[TCAA] (μg/L)	[DCAA] (μg/L)
7.4211	26.4103	5.5208	18.5816	10.4144	20.2310
6.6114	24.4680	4.9712	17.3412	3.6201	5.4152
8.0978	28.0586	5.0598	17.7292	5.6974	12.1682
8.8094	27.4765	6.2840	26.8559	4.931	9.7745
9.6251	28.5965	6.3831	23.5607	5.0157	11.5025
10.7835	32.0179	5.5436	21.1500	5.243	14.1802
6.8913	33.3070	8.0738	22.5156	7.2820	10.2585
6.7723	32.5835	7.4004	20.9489	4.5745	4.7755
9.6192	42.8934	5.9961	17.8214	7.9970	9.7765
6.3708	17.9684				
6.1431	18.5257				
7.9179	24.6553				
6.0118	15.3845				
5.0027	11.8707				
7.1730	16.2848				
8.9067	34.8010				
7.8758	30.1688				
7.9597	30.4527				
6.5423	19.6777				
6.3642	23.0130				
6.9947	24.4133				
7.5188 ± 1.4176	**25.8585 ± 7.5118**	**6.1370 ± 1.0429**	**20.7227 ± 3.2116**	**6.0861 ± 2.1070**	**10.8980 ± 4.6143**

Table A10: Summary of TCAA and DCAA concentrations in coffee, tea, and iced tea samples prepared with tap water. Averages and standard deviations are supplied at the bottom of the table.

Sample	[Residual chlorine] (mg/L)	[TCAA] (μg/L)	[DCAA] (μg/L)
MQ	N/A	0.3308	0.4892
MQ		0.2667	0.5709
MQ	0.05	0.1089	0.3329
MQ	0.03	0.1467	0.5149
MQ	0.05	0.0379	0.2027
MQ	0.11	0.1161	0.3008
MQ	0.06	0.0648	0.3538
MQ	0.02	0.0863	0.2027
MQ	N/A	0.0379	0.2027
MQ	0.09	0.0565	0.3008
MQ		0.0780	0.3538
Averages	**0.06±0.03**	**0.1210±0.0950**	**0.3477±0.1287**

Table A11: Summary of TCAA and DCAA concentrations in Milli-Q water. Residual chlorine concentrations detected in the water are provided where available.

Coffee		Tea		Iced tea	
[TCAA] (μg/L)	[DCAA] (μg/L)	[TCAA] (μg/L)	[DCAA] (μg/L)	[TCAA] (μg/L)	[DCAA] (μg/L)
0.8857	7.2142	0.2413	1.7886	0.1242	0.9643
0.9953	3.8282	0.0970	4.2234	0.1181	1.4782
1.6617	15.4522	0.0852			0.8743
0.2822	3.2180	0.1452			
0.5438	5.0844				
0.8117	3.1886				
0.1894	3.3713				
0.2278	3.2071				
0.1329	4.5652				
0.0977	4.3147				
0.5828 ± 0.5043	**5.3444 ± 3.7613**	**0.1422 ± 0.0710**	**3.0066 ± 1.7217**	**0.1212 ± 0.0043**	**1.1056 ± 0.3258**

Table A12: Summary of TCAA and DCAA concentrations in coffee, tea, and iced tea samples prepared with Milli-Q water. Averages are included at the bottom of the table.

Samples	[TCAA] (μg/L) (Experiment 1)	[TCAA] (μg/L) (Experiment 2)
1	0.0804	0.0682
	0.0607	0.1344
2	0.0869	0.1099
3	0.1154	0.1175
4	0.0434	0.0902
5	0.0898	0.0960
6	0.0529	0.1243
7		
Average	**0.0756**	**0.1058**
Standard deviation	**0.0249**	**0.0226**
MDL*	**0.0782**	**0.0710**

Table A13: Method detection limit data for TCAA concentration using SPME-headspace analysis of spiked water samples. TCAA samples were spiked to a concentration of 0.1μg/L prior to analysis.

*- The Method Detection Limit (MDL) is calculated using the formula MDL = *s.d.* X 3.143 (student *t* value for one tailed distribution with 6 *df* at the 0.99 confidence level).

Sample	[DCAA] (μg/L) (Experiment 1)	[DCAA] (μg/L) (Experiment 2)
1	1.7288	1.0764
	1.1331	0.5210
2	1.5875	0.8843
3	1.7756	1.2011
4	1.2133	0.4590
5	1.2366	0.9589
6	1.5228	0.7472
7		
Average	**1.4568**	**0.8354**
Standard deviation	**0.2613**	**0.2762**
MDL*	**0.8212**	**0.8681**

Table A14: Method detection limit data for DCAA concentration using SPME-GC analysis of water samples spiked with approximately 1.0μg/L DCAA.

Sample	TCAA concentration (μg/L)
HPLC water	0.042
HPLC water	not detected
HPLC water	not detected
Coffee	0.0781
Coffee	not detected
Coffee	0.0607
HPLC water	0.033
HPLC water	0.052
HPLC water	not detected
Coffee	0.0766
Coffee	0.0298
Coffee	0.0582

Table A15: TCAA concentrations detected in HPLC grade water (containing no residual chlorine concentration) and coffee prepared with the same water. All values obtained were below the MDL, and therefore do not provide valid experimental results.

Sample	Residual Chlorine Concentration (mg/L)
1	2.16
2	2.17
3	2.15
4	2.13
5	2.13
6	2.13
7	2.14
Average	**2.14**
Standard Deviation	**0.02**
MDL	**0.06**

Table A16: MDL results for the Hanna Hi-Range residual chlorine tester. Water samples used were Edmonton tap water.

Sample Date	Tap water [Cl] (mg/L)	Rossdale plant [Cl] (mg/L)	Variance	% Change
08/29/00	2.21	2.85	(0.64)	29%
09/11/00	2.27	2.94	(0.67)	30%
09/19/00	2.32	2.84	(0.52)	22%
09/28/00	2.19	2.66	(0.47)	21%
10/03/00	2.22	2.47	(0.25)	11%
10/19/00	2.15	2.70	(0.55)	26%
10/20/00	2.30	2.67	(0.37)	16%
10/23/00	2.23	2.65	(0.32)	14%
10/26/00	2.19	2.63	(0.44)	20%
11/01/00	2.28	2.60	(0.32)	14%
11/08/00	2.38	2.57	(0.19)	8%
11/14/00	2.28	2.64	(0.36)	16%
11/23/00	2.24	2.59	(0.35)	16%
Averages	**2.25 ± 0.06**	**2.68 ± 0.13**	**0.42 ± 0.15**	**19% ± 7%**

Table A17: Comparison of total residual chlorine concentration in tap water using the Hanna Hi-Range chlorine detector with the values detected at the Rossdale water treatment plant (information supplied by Epcor).